

How to Cut Your Own Throat

THE National Union of Teachers, one of the most conservative of British institutions, seems to be bent on making a mess of the teaching profession. At Scarborough last weekend, delegates to the union's annual conference instructed their officials to resist a scheme by means of which local authorities, supported by the government, would introduce a pay structure for teachers allowing those who make a career of teaching in the schools to be paid on a higher scale than those who have only recently entered the profession. Instead, the union is now committed to the principle of a single scale for teachers' pay which includes increments of salary for the first few years of service but which makes no formal distinction between the good teachers who have given decades to their craft and the fly-by-nights. As a result, it will remain necessary for local authorities to invent an even more wonderful spectrum of extra payments, special responsibility allowances and the like, so as to keep good teachers on their books. Even so, it will remain a problem to know how to pay a headmaster a salary likely to induce him to take risks. And there will remain the endless drift of exceptionally good teachers from the schools into other professions—educational administration among them—in which the rewards of exceptional talent are more conspicuous. The fact that the teachers are asking for an increase of 15 per cent, the chief reason why their negotiations with the management are at present deadlocked, is less important than the principle which their insistence on the single basic scale involves.

The first thing to be said is that the most serious flaw in the salary structure of British teachers is not at the bottom end of the scale but the top. Theoretically, the salary scale now in force runs from £1,050 a year to £2,000 a year. On the face of things, in other words, teachers beginning their careers are paid at rates quite comparable with those in similar professions. In April 1970, for example, the pattern of earnings among male teachers was such that ten per cent of them earned less than £22.3 a week. According to government statistics, the comparable figure for all academic jobs, in higher education as well as in schools, was £22.9 a week. To be sure, engineers, scientists and technologists are paid more handsomely at the start of their careers—the lowest decile is marked out at £24.4 per week—but all these professional groups earn more at the beginning of their careers than the £17.8 per week which distinguishes the lowest ten per cent of all full-time male workers from the rest. From this point of view, the most serious defect in teachers' pay is at the top and not the bottom. For male teachers in 1970, the median weekly earnings were £36.3 per week, but the upper decile in the wages pattern began at £47.6 per week. In other words, after a comparatively rapid rate of increase of pay, teachers can expect to settle down on a plateau in which their expectations must be rather carefully restrained. By comparison, academic workers as a whole, or scientists but especially those who work in industry and commerce, can expect much more rapid improvement prospects. It is all very well for the

new general secretary of the NUT, Mr Edward Britton, to make a populist appeal to his members by saying that it will now be possible for lavatory attendants at British engineering factories to earn more than teachers at the beginning of their careers, but this is quite beside the point as well as a sign of the snobbery to which teachers are prone. By what right, after all, should Mr Britton suppose that beginning teachers should always be paid more than diligent workers elsewhere who carry out tasks which teachers—or at least Mr Britton—despise?

Two issues arise. First, there is the practical question of how the government and the local authorities should try to visit on the teaching profession a salary structure likely in the long run to be in the best interests of the teachers as well as of the schools they serve. The most urgent need is to simplify the present arrangements under which able teachers collect several different kinds of allowances until they find themselves unable to move easily from one school to another for fear of not being able to carry their extra salary with them. What the government is seeking is to replace this cumbersome system by one in which there would be a uniform scale of extra payments throughout England and Wales. The difficulty, of course, is that there is bound to be this year such an argument about the amount of the pay increase that it is hardly to be counted as practical politics that there should be such a radical structural alteration.

But what is to prevent individual employers, in this case local authorities, from introducing supplementary scales of the kind which the National Union of Teachers has set its face against? Such a development could, after all, help enormously to give the teaching profession a sense that there are tangible as well as intangible benefits to working for readily identifiable employers. For the local authorities, such a development would provide a greater sense of mobility among the teachers on its books, and it would also be possible for progressive authorities to attract good teachers simply by paying a little above the odds. It is true, of course, that the change would be a break with the tradition that local authorities as a whole should negotiate with the teaching profession as a whole, but this is hardly an essential part of the process of fixing scales of pay. Indeed, in the present climate it is almost a disadvantage to have one single negotiation to determine the pay of every teacher—even at the most mundane practical level, the arrangement serves chiefly to ensure that the price of failure in the negotiations will be almost intolerably high. It goes without saying that a development like this would also have the advantage of challenging the National Union of Teachers with the need somehow to prevent itself from being overtaken by the much smaller union, the National Association of Schoolmasters, which has won notoriety for its clever public relations and for its old-fashioned views on equal pay for women, but which has had the merit of recognizing the importance of a more definite career structure.

The second issue which seems to have been largely



ignored at the NUT conference a week ago is that of what kinds of jobs teachers can expect to be doing a decade or more from now. Whatever happens, it is important that the future will be unlike the past. Indeed, some of the most encouraging developments in school education in the past few years have contained the seeds of qualitative change in teaching. To begin with, it is now clear that schools must be the principal instruments of curriculum development, which implies that even in a climate of education for all, a significant proportion of those who teach in schools must be academic innovators. Second, new educational techniques, from team teaching to television, entail that many teachers will in future have to function as managers as well as pedagogues. Often it would be in the best interests of the students in the schools if the teaching profession were organized in such a way as to make full use of incidental help—teaching ancillaries both human and mechanical—but this is another issue on which the National Union of Teachers takes a predictably conservative and stuffy line.

But no amount of collective foot dragging will allow the teachers to head off unavoidable changes—they can

merely help to ensure that the full benefit of innovation is to some degree truncated. The truth is, however, that in the decades ahead, the differences of quality between skilled teachers and the raw recruits will become more and not less apparent than they are at present. To a large degree, the traditional belief that a British teacher's classroom is his castle will have to be abandoned in favour of a system which allows the skills of experienced teachers to be spread more widely than at present. Although such diversity within the profession could in principle be accommodated within the present system of allowances for special responsibility added to teachers' basic pay, the changes in prospect go too deep for that strategy to be effective. Indeed, the years ahead promise to be so full of qualitative change that there will be a need to create not merely new salary scales but new categories of people. The objective should be to make the fullest use of people who, without recognizing it, more than any other professional group hold the future of society in their hands. This is yet another reason for deploring the level on which the teachers' consideration of the future of their profession is now conducted.

Smoke without Fire

In the past few weeks, there have been cheerful reports from the United States that the question of a comprehensive test ban treaty is being resurrected. Although there seems to have been no decisive change in the technical framework within which such an agreement might be policed effectively without the need for on-site inspection—the issue which has consistently brought attempts at a comprehensive test ban treaty to frustration—there does appear to have been a change in the attitudes which nations take towards the necessity of a treaty which is for practical purposes cast iron. This, it seems, is the spirit in which the United States government has decided to raise the question of a comprehensive treaty in the disarmament committee at Geneva.

What seems to be happening is that the climate has benefited enormously from the inquiry last year by the Secretary-General of the United Nations about the capacity of member nations to help in the provision of seismic records of the kinds necessary to monitor a comprehensive test ban treaty. What has clearly emerged from the UN survey is that the installations for recording seismic disturbances which are scattered about the world are at once more numerous and more sensitive than had previously seemed to be the case. The whole, it seems, is larger than the sum of the parts. But it is also clear that the instruments now installed at some seismic stations are every bit as sensitive as they were hoped to be, while it becomes apparent that sufficiently sensitive equipment can realistically distinguish between explosions underground and natural earthquakes, at least if the explosions are big enough. It may never be possible with speed and precision to identify as such a nuclear explosion of a kiloton or two in soft rock at a distance of a thousand kilometres, but it begins to appear that by the end of the decade, the remote detection of the kinds of explosions likely to be of military use will be a practical proposition. That is a big step forward.

It follows that there is now a need for a comparable

change in the attitude of the governments which would have to sign a comprehensive test ban treaty. In the early sixties, when the issue was last aired in public at a high level, it became apparent that the Soviet government would have nothing to do with a treaty that would provide other nations with the right to inspect installations on its territory. To be fair, it must be acknowledged that the United States Congress was never required to agree to a corresponding abandonment of sovereignty. What Congress did insist upon, however, was that any comprehensive test ban treaty would have to provide some mechanism for making sure that suspicious events in the Soviet Union (or elsewhere) were earthquakes and not explosions, and there was talk of how to carry out on-site inspections in such a way as to ensure that the cause of a suspicious seismic disturbance was not an underground explosion. In circumstances in which there might be hundreds of potentially suspicious events each year, such an anxiety is entirely understandable. Because the distinction between earthquakes and explosions is necessarily least precise when the seismic disturbance is least intense, it is inevitable that the increasing sensitivity of the instruments should throw up each year a larger number of events which cannot unambiguously be ascribed to earthquakes, not explosions. But who cares if one side or the other should think it possible to mount a military development programme on the basis of underground explosions each no larger than the equivalent of a few kilotons? And in any case, does not even such a programme entail the risk of accidental detection? These are the questions which politicians have been asking themselves in the past ten years. The point seems to have been reached when many of them are prepared to regard a nation's right to abrogate a treaty as the equivalent of all kinds of on-site inspection.

So why not negotiate and sign a comprehensive test ban treaty right away? The trouble is that in the past few years, both the United States and the Soviet Union seem to have been forced to recognize that nuclear weapons

programmes are still essential parts of their military research and development. So long as most nations are prevented by the non-proliferation treaty from acquiring materials with which to make weapons, while Britain seems to have lost interest in nuclear testing and while only France and Mainland China conduct modest programmes, it seems quite safe for the two super-powers to push vigorously ahead with the development of anti-ballistic missiles, multiple warheads and even the improvement of existing military nuclear explosives. The snag, as both of them are now beginning to discover, is that it is virtually impossible to reconcile such programmes with the need which both powers recognize to create means of restraining their mutual destructive capacity as in the SALT negotiations. And, of course, it is becoming plain that an efficient test ban treaty could go a long way to do what is likely to emerge from any agreement in Vienna. At the very least, it would tie both nations' hands behind their backs. In circumstances like these, of course, there is theoretically a danger that one side or the other might be able to steal an advantage, either by a premeditated abrogation of the treaty or by sheer cleverness and stealth, but these are exceedingly improbable calculations. What seems to be happening now is that the super-powers are beginning to see the errors of their previous ways.

It goes without saying that the benefits of a comprehensive test ban treaty would outweigh those of even the considerable measures of arms control which have been

implemented in the past few years or which seem just over the threshold—the non-proliferation treaty itself, the agreements on nuclear weapons in the Antarctic and in outer space, the discussions on the demilitarization of the sea bed and the various ways which have been proposed for strengthening the Geneva Protocol on chemical and biological weapons. In particular, a comprehensive test ban would help to get rid of the asymmetry built into the non-proliferation treaty, which gives the major powers pride of place. (Nations which have bombs already can continue to develop them, but nations without bombs must stay that way.) It would also be an improvement on the partial test ban, which forbids the testing of nuclear weapons in the atmosphere, especially as it is now becoming unclear what significance should be attached to underground explosions which vent radioactivity to the atmosphere. But then, to the extent that a comprehensive test ban treaty would limit the capacity of the super-powers to develop new kinds of warheads, it would subsume agreements such as that on the demilitarization of the sea bed. But finally, there is the more mundane but equally telling consideration that a test ban treaty, by removing the need to verify that the non-proliferation treaty is being honoured by its signatories, would allow the International Atomic Energy Agency to abandon a programme of inspection (among the nuclear have-nots) that threatens to become as much of an international hazard in its own right as any violation of the treaty.

Weather Watching

WITH all the fuss there has been in recent months about the possibility that carbon dioxide in the atmosphere may affect the climate on the surface of the Earth, it is something of a surprise that so little attention is being paid to the long-term climatology which must, most people would suppose, form the basis for objective assessment of the effects of recent changes in the constituents of the atmosphere. Even in the United States, where the concern about what is called the greenhouse effect appears to be strongest, the research programmes being undertaken by various federal agencies are more concerned with such things as the detailed measurement of dust particles in the stratosphere or the construction of mathematical models to predict the consequences of changes which may be brought about. By comparison, comparatively little effort has been devoted to the long-term historical studies which could, in principle at least, help to throw light on problems of immediate concern. After all, the fluctuations which there have been in climate in the past two centuries, many of them now well documented, are often greater than those expected to be caused as a result of atmospheric contamination of the most serious kind. Going further back, the fluctuations in the centuries immediately following the melting of the ice 8,000–10,000 years ago were—perhaps understandably—even more dramatic. In all these circumstances, it is a great surprise that simple climatology is so neglected. After all, in the nature of things, the benefit of more research of a quite orthodox kind could easily be not merely some measure of understanding of recent fluctuations of climate but also an answer to the question whether the Earth's climate is

now comparatively stable or whether the present period is merely an interstadial, an interval of recession between two ice ages. Whatever the Jeremiahs may say about the dangers of climatic change brought about by carbon dioxide, there is no question that the return of the ice would be a greater calamity. To be sure, while offering few immediate short term advantages, prior warning of such an impending disaster might prove of no small practical value. And that is a powerful case for more climatology.

100 Years Ago



NOTES

A PROPOSAL has been made that certain Medical Schools on the north and south sides of the river should be amalgamated, in order that, by concentration of power, the teaching shall be made more efficient than it is at present, the teachers being able to devote themselves more unreservedly to their duties than they possibly can do under existing arrangements. The absolute necessity of some such arrangement as this is obvious.

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OLD WORLD

FRANCE

Modest Ambitions

Paris, April

Now that the dust has settled on the programme for the VIth quinquennial research and development plan, it is clear from the forecast of the money to be spent between now and 1975 that moderation must be the order of the day. The planners may have come a long way since the first guidelines were laid down in 1968, but they seem to have finished the journey in reverse gear.

Two years ago, the Consultative Commission for the plan decided that by 1975, 3 per cent of French resources should be spent on research and development, a figure based on the economic concept of total *production intérieure brute*, a sum which is about 10 per cent less than the more familiar GNP. Towards the end of 1969, M Jacques Chaban-Delmas, the prime minister, accepted the 3 per cent objective, but by last autumn this had begun to hover between the 2.55 per cent and 2.70 per cent marks. At present, the figure is holding firm at 2.50 per cent, a goal identical with that projected under the Vth plan and which, ominously, was not attained. To reach the 2.50 per cent target, the growth rate of investment in research and development was estimated only a few months ago to be 13 per cent, with 11.70 per cent as a lower limit: now it seems that the figure will be a trifle under 11 per cent.

What will the modesty of these prospects mean for French science? Clearly, major programmes of research in science and technology are a thing of the past, and it seems likely that current large scale enterprises may be pared down. Nevertheless, 4,000 million francs (£299,500,000) have been earmarked over the next five years for electricity produced from nuclear energy, and the same amount for the space programme. Research activity destined for industrial application will be augmented by a minimum of 10,980 million francs, reaching as much as 11,230 million francs (£841,000,000) over the amount spent during the period 1966–70.

Whereas there had been some talk of increasing the total number of jobs in the scientific civil service, any growth will now be at a modest rate. "The large expansion foreseen in industrial research," on the other hand, "should mean considerably more openings" in private enterprise. A "sub-

stantial" effort will be made in the category called *l'aide au développement*, a strings-attached arrangement whereby state loans proffered for industrial development must be repaid if the project proves successful and profitable. This aid will amount to 2,000 million francs, or about 9 per cent of the gross French outlay of more than £1,600 million during the coming five years.

Grants for research and development which will be conducted strictly within the confines of private industry will total 3,300 million francs, about 15 per cent of the scheduled vote, and certain tax incentives will be offered to those forms clearly involved in research activities. Life sciences and the socio-economic sciences (city planning, housing and transport) should enjoy a considerable boom, however, for they have been awarded some 10 per cent of the research and development budget, a total of between 2,195 and 2,235 million francs (roughly £165,917,000). On the other hand, research devoted to conservation and improvement of the environment will amount to 600 million francs (more than £44,100,000).

The precise manner in which the financial cake will be cut up between the various sectors of pure and applied research remains to be decided, and, of course, the entire plan awaits the final approval of the Minister of Finance and Economic Affairs, M Valéry Giscard d'Estaing, and the French Parliament towards the end of the current parliamentary session.

UNIVERSITIES

Dainton Swing Slows

THE most recent volume of educational statistics published by the University Grants Committee does nothing to quiet the anxiety which has troubled the scientific community ever since the Dainton report of three years ago underlined the full extent of the swing away from science in the sixth forms. Comparative figures from the report (*Statistics of Education*, 1969, Vol. 6: Universities, HMSO, £2.70) indicate the inexorable progress of that trend. In 1965–66, 58.1 per cent of the student population of British universities were engaged in studies which the UGC describes as science based (meaning all those topics which cannot be included under the headings of education, social, administrative and business studies, or language, literature and arts other than languages). By 1969–70, this proportion had fallen to 54.7 per cent, and during this period the percentage of students studying pure science was within a few per cent of the number engaged in social, administrative and business studies.

Nevertheless, Mrs Margaret Thatcher, Secretary of State for Education and Science, was characteristically optimistic when she suggested recently that there were signs that the swing away from science in the schools had been slowed and perhaps even halted. Speaking on the first day of a conference to celebrate the centenary of the Mathematical Association, Mrs Thatcher observed that the numbers of students electing to study mathematics in the sixth form were increasing, not only in terms of actual figures, but as a percentage of the entire student population. This trend had, she felt, important implications both for the teaching and practice of mathematics in Britain and for the future of science as a whole.

Predictably enough, the number of students in full-time university education in Britain rose to a new record high in 1969–70, with a total of 219,506, compared with 211,485 the previous year. Of this number, 28.1 per cent were women, thus continuing the slow upward trend; in 1959–60 only 24 per cent of the student population were women. No strong upward trend could be seen in the number of teaching and research staff. The total was 26,067 in 1968–69, but rose to only 26,904 in 1969–70. Of this number, some 11 per cent were of professorial status, 19.5 per cent readers and senior lecturers and 65.4 per cent lecturers and assistant lecturers.

The total income for the universities in the year 1968–69 was £236,283,095, of which 71 per cent was accounted for by Exchequer grants, the bulk of the remainder coming from fees (7 per cent) and governmental payments for research (7 per cent). Some £8 million, or about 3 per cent of the total income, was derived from repayment of Selective Employment Tax.

FURTHER EDUCATION

Planning in Chaos

by our Education Correspondent

AN attempt to provide higher education on the cheap lies behind the abrupt dismissal last December of two part-time lecturers at the Bolton Institute of Technology and the reduction in the teaching hours of at least eight others. That is the opinion of a team of investigators from the Council for Academic Freedom and Democracy, which has recently published a report on the dismissals (*The Bolton Dismissals*, available from the National Council for Civil Liberties, 12p). But the root cause of the problem goes much deeper than the events themselves suggest—it is firmly embedded in the chaotic method of academic planning in the further education sector and the system by which many colleges are chasing

after university or polytechnic status. As the council says in its report, "any censure of ours must be directed at the system in which colleges feel obliged to act immorally even to bring provision for existing courses up to scratch, let alone to extend them".

The dismissals themselves took place on December 7, 1970, some six weeks after the registrar had questioned the expenditure on part-time teaching salaries during a meeting of heads of departments. Two specialist teachers from the Department of Liberal and Social Studies and Modern Languages were given four days' notice and the department was left without a native French speaker among the French staff and in a situation in which two lecturers were forced to teach 45 students the whole range of English literature. The upshot was, as the council points out, that "the morale of both staff and students has been badly hit".

What seems to have happened is that Bolton Institute of Technology has been concentrating on high level courses in an attempt to attract more students and to gain academic respectability. In so doing, it has over-extended itself and has been caught up in a cost spiral. But this is a familiar story in the further education sector, where the carrot of degree-level courses and their related kudos has led many colleges to have university aspirations. The resulting vicious circle, the Council for Academic Freedom and Democracy suggests, is that "an increase in facilities and staffing will only be possible if the student intake is expanded, although an expansion of intake is only morally justifiable if facilities and staffing have been increased".

But that is only part of the problem. In addition to the drive for academic status, the colleges are also servants to two masters as far as academic planning is concerned. The Department of Education and Science is responsible for recognizing courses, and if it withholds that recognition, the local authority would have to shoulder their cost. Moreover, degree-level courses must be validated by an external authority such as the University of London or the Council for National Academic Awards. This situation, the council report suggests, is rather like a game of three-dimensional chess.

This interplay between the various planning factors has already resulted in one bizarre situation at the Bolton Institute of Technology, and the implication in the report is that it is responsible for much of the disquiet that lies underneath the surface. The particular situation in question is the setting up of a course in psychology, leading to an external degree from the University of London. The Department of Education and Science refused to give

approval for the course, but the institute nevertheless recruited students and staff for it. The department's hand was forced, and approval was given for only one year's intake—a situation in which the education committee was indisposed to sanction large amounts of money for equipment and books. The students consequently arrived expecting a psychology laboratory, but found instead a room marked laboratory with precious little in it.

The only effective way to relieve the pressure between institutions and to prevent such blatant status-seeking, in the opinion of the Council for Academic Freedom and Democracy, is to set up a body on the lines of the University Grants Committee but concerned with the whole of higher education. The alternative is the perpetuation of the binary division between the universities and the colleges.

INDUSTRIAL TRAINING

Recipes for Change

THE rumbles of dissatisfaction at the way in which the Industrial Training Board system operates are likely to be reflected in the review now being made by the Department of Employment. In particular, it seems almost certain that the arrangement whereby the levies from firms are returned in the form of grants for suitable training schemes will be transformed so that levies can be reduced and the advisory nature of the boards expanded.

The training boards were set up under the 1964 Industrial Training Act, and now number twenty-eight. They are expected to make levies of about £175 million a year. The largest of them, the Engineering Industry Training Board, has a levy approaching £80 million a year and has responsibility for more than 3 million of the working population. (Not all of this levy is collected, for companies with training programmes keep a large part of the total.)

Much of the criticism of the boards has been crystallized by the Confederation of British Industry, which has put forward several suggestions which only just fall short of a recommendation that the training boards should be disbanded. The CBI advocates a gradual shift of emphasis, from the grant and levy concept, towards the concept of a training board as a body responsible for the long-term planning of training and for manpower forecasting. The last of these tasks was, in fact, one of the original objectives in the Industrial Training Act, but it does not come high in the list of achievements of the boards.

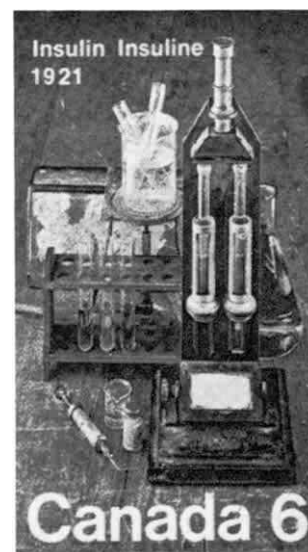
The CBI would be the first to admit

that levies cannot be reduced too quickly without losing the benefits of the past few years. Another of the problems which the Department of Employment will no doubt be considering in its review is that of capital expenditure by the training boards. Most firms are understandably sensitive about the use of money (which they have contributed) for projects, such as the building of research centres, whose benefit may have been inadequately defined.

SCIENCE ON STAMPS

Hormone to Remember

A RECENT Canadian stamp commemorates the 50th anniversary of the discovery of insulin. This, the internal secretion of the islets of Langerhans in the pancreas, is a hormone enabling the tissues requiring sugar for their activity to absorb it from the bloodstream. With a deficiency of insulin, sugar accumulates in the blood and is excreted continuously or intermittently into the urine, chief symptom of the disease known as diabetes. It was the discovery of insulin by the Canadians McLeod, Banting and Best, late in 1921, which was to remove diabetes from the list of fatal diseases, their work resulting in the awarding of the Nobel Prize for medicine to McLeod and Banting in 1923.



1.5 times actual size.

The stamp, Canada's first four-colour photographic example, shows the colorimeter used by the discoverers in their experiments, the earliest vial of insulin, an old hypodermic syringe which might have been used to administer insulin fifty years ago, some test tubes intended to represent tests for glycosuria and a pancreas preserved in a museum jar.

EDUCATION

Astronomy to Turn Tide?

PROPERLY taught astronomy courses may offer the best hope of stemming or even reversing the drift away from scientific subjects at all levels of education. At a meeting of the Royal Astronomical Society, held last week, a group of senior astronomers under the chairmanship of Professor D. McNally discussed ways in which astronomy can and should be taught, in schools and at both undergraduate and postgraduate level in universities. Professor A. J. Meadows, of Leicester University, emphasized the different approach required for teaching general astronomy courses rather than those aimed specifically at the few students intending to take up a professional career in the subject. Many of the participants at this meeting could not, however, agree with Meadows's suggestion that below graduate level no one could honestly be called an astronomer—if only because of the prospect of losing potential astronomers to other more specialized disciplines. It was felt that even introductory courses should be honoured with the title "astronomy".

But there was a much more general agreement on the value of astronomy as a subject for the non-science specialist, or for the specialist in another branch of science who may become a teacher. In schools, particularly, there is no doubt that astronomy can be a most attractive subject for students who otherwise profess no interest in science; this has encouraged the introduction of an astronomy O-level, and the prospect of an astronomy A-level. The professional astronomers were not, however, happy about this prospect, feeling that the introduction of astronomy and astrophysics to the mathematics and physics curricula, as in Nuffield physics, offers a better chance of attracting students through astronomy to science in general. Unfortunately, as Professor Roger Tayler of Sussex University was quick to point out, there are very few suitable books for the teaching of astronomy at this level, and many existing popular astronomy books perpetuate errors which later have to be unlearned.

Perhaps the most immediately interesting news came from Glasgow University, where it is once again being demonstrated that the Scottish educational system is at least one step ahead of the rest of Britain. Professor P. A. Sweet reported that the decline of popularity of science courses in Glasgow has resulted in the introduction of large amounts of astronomy to the undergraduate courses, and that this move has so far met with considerable success. The Scottish system, which

requires four years' training for an honours degree, provides more flexibility in first year teaching, enabling all students, in both Arts and Science faculties, to attend the same introductory classes. But the greatest success in astronomy teaching at Glasgow has come over the past year, with the introduction of a course training future professional astronomers and making ingenious use of laboratory simulation of observations, developed largely by Dr D. Clarke. By taking the pressure off of valuable observatory time, these laboratory experiments, using star simulators, have made it possible for the number of students to be greatly increased.

In general, it seems that practical astronomy courses of the kind available in Glasgow, or the option of "selected topics" covering certain theoretical aspects of astrophysics in depth during the final year of undergraduate courses in physics and mathematics, such as are now available in Cambridge and at Sussex, are meeting with an enthusiastic response from students. At a time when science in general is losing its attraction, yet we require an ever-growing number of trained scientists to run our technological civilization, this development should surely receive more widespread attention and support. It is debatable whether or not the Department of Education and Science will take notice of unsolicited advice, even from so august a body as the Royal Astronomical Society; but the time is surely ripe for an approach to be made, and if this does not come from the government then it must be the responsibility of the RAS to initiate proceedings which may have a profound impact on our educational system.

COMMUNICATIONS SATELLITES

Symphonie and Intelsat

Paris, April

ON the heels of the announcement that more than 2,000 man-made objects are in orbit round the Earth and that another 2,700 have fallen back and disintegrated, the Franco-German Symphonie communications satellite project is in full swing. Technically, all is ready. Symphonie recently got the green light from General Aubinière, Director-general of the French Space Research Organization CNES, Dr Mayer of the West German space unit and a representative of CIFAS, the Franco-German industrial consortium for Symphonie. The contract will cost France £12,210,000 and West Germany £14,157,000. Two operational models of the satellite are due for delivery on June 19 and October 30, 1973, to the

French and Germans respectively. The prime contractors will be Aérospatiale of France (at Les Mureaux) and Messerschmidt-Boelkow Blohm (at Munich).

The two governments signed the original Symphonie agreement on June 6, 1967. The idea derived from the earlier German Olympia and French Saros concepts, adapted in March 1969 to new objectives. The recent agreement ensures that the project will be carried through to the construction and launching of two Symphonie satellites in about two years time. Although strictly a Franco-German initiative, the project could have an important influence in Europe and elsewhere.

With the American Intelsat programme probably more than adequate to meet German and French needs, it is a question to know why Symphonie was thought to be necessary. Paris and Bonn have both expressed a preference for a measure of European independence in satellite communications, chiefly because of their concern about the possibility of foreign monopoly control over television programmes. Thus Symphonie's first real mission will be the preservation of at least limited European freedom in this domain. As opposed to satellites with world-wide coverage such as those of the Intelsat series, Symphonie will retain a more regional characteristic, basing itself on a number of smaller stations of modest antennae capacity. This feature should prove to be attractive to countries with relatively low density telephone networks, especially in Africa and the Middle East. It remains, however, to convince such countries that participation in the commercial exploitation of Symphonie will be economically sound. In some cases this may not be easy; the Ivory Coast, Senegal, Cameroon and Madagascar have already subscribed to Intelsat.

There will also be the problem of persuading Comsat, the American organization which is the manager of Intelsat, that Symphonie will be compatible with Intelsat. To that end, the French and Germans are trying to have Symphonie accepted as part of a regional broadcasting service for television as well as the precursor of the telephone distribution system for the African continent.

Regardless of how Symphonie is finally used, launching in 1973 will be from Kourou in Guyana using Europa II boosters. The satellites will be in the geostationary orbit and will handle primarily telephone circuits beamed toward a Euro-African zone and, secondarily, television circuits to and from North and South America. Should one of the two Symphonie communications packages break down, the other could immediately be used in its place.

NEW WORLD

Social Scientists still Poor Cousins

by our Washington Correspondent

"It is viewed now as 'natural' that the head of NSF be a natural scientist; that the Office of Science and Technology in the White House will be dominated by natural scientists; that the scientific academies will include only a few social scientists, and so on. But now, as our prime business shifts, physics and chemistry must learn to take a second seat to other sciences, especially biology, physiology and the social sciences"—Amitai Etzioni, chairman of the Department of Sociology at Columbia University, addressing the American Physical Society, New York, February 1, 1971.

If complaints such as Etzioni's seem tinged with bitterness, that is because for years social scientists have not only been relegated to inferior academic status—the National Academy of Sciences elected its first social scientist as recently as 1966—but they have also been far outdistanced by natural scientists in the competition for influence and funds in Washington. With the recent shift in national priorities from defence and space to the needs of the community, social scientists are expecting their share of research funds to grow in proportion. But there is little sign yet that the extraordinary increments in research funds that biologists and physicists were able to arrange in their heyday of power will be as easily obtained by the social scientists.

One bad omen is that Congress, in considering the shift in national needs, has seen as its first priority not the pouring of money into the coffers of social science departments but the retraining of out of work aerospace scientists and engineers in socially relevant skills, including many that are the prerogative of social scientists. Another obstacle on the road to riches is the uneasy relationship between the social science community and the Department of Defense. The department accounted for nearly a third of all federal government support of research in 1970, assigning \$122 million to the life sciences, \$30 million to psychology, \$355 million to the physical sciences, \$186 million to the environmental sciences, \$107 million to mathematics and \$1,009 million to engineering. The social sciences received only \$7 million of defence money.

Project Camelot, which blew up in the army's face in 1965, is one reason

for the Pentagon's sparing support of social research. The project, a study of social change in Latin America, was assailed as a form of US intervention, caused grievous diplomatic embarrassment, and was cancelled by order of President Johnson. More recently, the Mansfield Amendment, requiring the Department of Defense to support only research with a direct relevance to military need, has served to restrict the department's involvement with the social sciences. Another inhibition to close relations between the military and the social science communities is that some social scientists who are opposed to the Vietnam war take the view that the Pentagon does not deserve the benefit of their services.

Federal Obligations for Research in Social Sciences (in millions of dollars)

Year	1968	1969	1970
<i>Department or agency</i>			
Defense	6	8	7
Health, Education and Welfare	76	83	84
Housing and Urban Development	4	2	5
Agriculture	30	31	33
Commerce	12	10	12
Labor	9	9	9
State	3	3	4
Transportation	5	13	18
National Science Foundation	17	17	20
Office of Economic Opportunity	17	21	20
Others	16	16	20
Total	195	213	232

An interesting attempt at mediation between social scientists and the Department of Defense has recently been made by a committee of the National Academy of Sciences*. The two chief recommendations of the committee are, first, that the Defense Department should develop a first rate in-house capability in the social and behavioural sciences and, second, that research in other countries ("foreign area research") should be handed over by the department to a "government-wide institutional structure". It must be a rare occasion when a recommendation of an academy committee is imple-

* *Behavioral and Social Science Research in the Department of Defense: A Framework for Management*. National Academy of Sciences, 1971.

mented instantly by the government department concerned, let alone when several parts of the bureaucracy are involved. But this has apparently happened to the academy committee's advice on foreign area research. Last week the State Department announced that a new inter-agency group would be set up to coordinate federal research related to foreign affairs. (The new group is in fact the result of another government committee set up by the National Security Council.) The inter-agency group, known as the Under Secretaries Committee on Foreign Affairs Research, will be chaired by a State Department official, with members from the Defense Department, the US Information Agency, the Agency for International Development, the Arms Control and Disarmament Agency and the National Security Council. The President's Science Adviser, Edward E. David, has observer status on the committee, as does a representative from the National Science Foundation. The State Department's budget for external research is smaller than that of any other agency concerned with foreign affairs; to have won control over the new coordinating committee represents a substantial restitution of its authority in this area and one that has long been urged by Senator Fulbright among others.

Institution of the new foreign research group may assist what is evidently one of the chief purposes of the National Academy committee, to overcome what is described as "the bitter feeling today between the Department of Defense and some segments of the social science community". In language chosen to be placatory to both sides, the committee describes the faults of each. Pentagon officials believe non mission-oriented basic research "to have lacked policy payoffs and to have constituted both a subsidy to producers (of the research) and a source of difficulty and irritation with the Congress. Research producers are sometimes viewed as being more interested in furthering their academic disciplines than providing operational help to the Department of Defense. . . . To its critics, much social science research has appeared to be simply fact-gathering unrelated to hypotheses and, when used, the hypotheses seem to be those generated by their relevance to the discipline rather than to consumers of research and the Department of Defense."

From the point of view of the researchers, the academy committee notes that many policy-makers are unable to pose their problems in researchable terms. The committee also makes the point that the Pentagon can and should learn from its critics—"The Department of Defense needs to expose its own thinking and assumptions to outside criticism and, in consequence, has an obligation to provide at least some outside critics with sufficient information about its assumptions to provide a basis for intelligent criticism." The statement is immediately balanced by the admonition that social scientists who are critical of United States policy "should not withdraw from the task of analysing national security policy merely because they are at odds with it or made angry by it. The appropriate institutional arrangement for critical research is probably one in which the Department contracts with an independent research institute. . ."

As a rationale for transferring foreign area research to an inter-agency group, the academy committee argues that knowledge is power and "as the possessor of the most knowledge the Department of Defense tends to be in a commanding position and therefore is not compelled to try to do the best possible job in analysing and defining any situation. It is difficult for other agencies to present and argue for a different picture in the absence of knowledge even approaching that possessed by the Department of Defense."

The academy report makes explicit its concern with the question of what social scientists can do for the Department of Defense, as well as the corollary question that was the almost exclusive concern of the previous academy report. How far the committee's conclusions will be acceptable to the social science community is hard to predict, but staff members of the National Academy draw comfort from the generally favourable comments at a recent annual meeting of the Division of Behavioral Sciences of the National Research Council, the operating arm of the academy. One view presented at the meeting was that things are so bad that Defense Department money should not be accepted on any terms, but according to observers this was a minority view and most of the 50 social scientists attending agreed with the academy committee's position.

Even if the social science community at large is equally ready to bury the hatchet, the Department of Defense has its own problems in reaching a new accommodation. The recent hearings on army surveillance of civilians held by Senator Erwin's Subcommittee on Constitutional Rights have created a distinctly hostile atmosphere towards any scientific or technological develop-

ment that might increase the Pentagon's power over the lives of US citizens. Symptomatic of this hostility is an interchange of letters between Representative Cornelius E. Gallagher and the Secretary of the Navy, John H. Chafee, which Gallagher read into the *Congressional Record* last month. Hearing of a Navy supported psychological study entitled "The Value of a Human Life: An Initial Analysis", Gallagher wrote to Chafee saying that as chairman of the House Privacy Subcommittee he found the fact that Navy funds were used to undertake such a study a source of great concern, "especially in a time when, rightly or wrongly, many citizens view actions of the federal establishment with increasing suspicion". In answer to questions raised in his letter, Gallagher received from Robert A. Frosch, Assistant Secretary of the Navy for Research and Development, a list of 110 reports published within the past two years of psychology research supported by Navy funds. Some of the titles, such as "How to Introduce Needed Change in Navy Organizations", bear a direct relationship to the Navy's functions; others, such as "Empathy Projection and Negation in Seven Countries" or "Values and Public Dissent—Preliminary Measures", are of less obvious interest to naval men. In one report entitled "Category Analysis of the Scoring System for Hostile Press" the term "hostile press" turns out to be jargon for a "tension-laden personal situation", which earned from Gallagher the observation that even the faint suggestion that the Navy is studying categorizing unfriendly newsmen is unfortunate in today's society. "I believe that the use of Navy funds to pay for this study . . . is extremely dangerous, especially in a time when military spying on civilians is so widely discussed," was Gallagher's final comment on the Navy's list of research projects.

Besides their uneasy partnership with the military, social scientists have also been at a disadvantage compared with natural scientists through a lack of any secure base for influencing government policy. The physical scientists have been able to operate through the Office of Science and Technology, the President's Science Advisory Committee and the Atomic Energy Commission. Biologists have worked through the National Institutes of Health. Social scientists have lacked any focus from which to construct coherent policies for their subject. This has not been for want of trying. When the National Science Foundation was being set up in 1946–50 there was an attempt to include the support of social sciences in its mandate. The compromise reached was that the NSF was allowed, but not required, to support the social sciences.

The oft-mooted proposal of a separate foundation for the social sciences came nearest to fruition with a bill introduced by Senator Fred R. Harris in 1968. The social science community was, as usual, divided about the proposal, which in the event came to nothing. Almost equally little has come of the suggestions of a National Academy committee which reported in the same year the need for assigning to the Office of Science and Technology responsibility for the social sciences. In OST and the President's Science Advisory Committee, the academy report stated, "There is a clear deficiency: the OST does not have staff competence in the behavioural sciences and until recently there were no behavioural scientists among the members of PSAC, the first having been appointed in 1968. The deficiency in OST is particularly significant because of the focal position played by the Office in policy formation and in staff studies for the President".*

The only real foretaste of impending largesse that the social scientists have yet received is a substantial increase for research support in the budget of the National Science Foundation. For the coming financial year the NSF is requesting from Congress \$27.5 million for the support of social science research projects, an increase of \$10 million over the budget for 1971. Some of the increment is offset by reductions in the institutional support the foundation is giving to social as well as other sciences, but a further instalment of up to \$10 million is in prospect from a new NSF programme entitled Research Applied to National Needs (RANN).

Despite the shift in direction of the NSF towards the social sciences, a tendency which is likely to continue for the immediate future, the natural scientists have shown little inclination to share the levers of power with their social science colleagues. Understandably perhaps, this has created some impatience, as well as being even detrimental, in the view of one critic, to the development of the social sciences. The Columbia University sociologist Amitai Etzioni told a meeting of the American Physical Society last February that "while the natural scientists have a significant role to play (in solving national problems), their predominance in the representation of all sciences to the policy makers and their controlling role within the scientific community, such as NSF and the academies, has resulted in a slowing down of the evolution of the social sciences and in restriction of their access to the needed financial support and to the ears of the policy makers, and hence has impeded the treatment of our social problems".

**The Behavioral Sciences and the Federal Government*. National Academy of Sciences, 1968.

UNIVERSITIES

Changeover at Illinois

from a Correspondent

DURING the past 12 months, 266 new college presidents have been appointed to positions at institutions, including Harvard and Massachusetts Institute of Technology, Wisconsin and Northwestern. Princeton, Purdue, Syracuse and the University of California at Berkeley are among those seeking new presidents now. A recent major appointment in the middlewest has been that of John E. Corbally, Jr, to the presidency of the University of Illinois.

Founded in 1867, the university now has three campuses and is known as the University of Illinois system. In addition to the Urbana campus, there is the large Medical Center on Chicago's West Side, located in one of the city's largest ghettos.

A third campus, the recently developed Chicago Circle skyscraper, stands just west of Chicago's downtown district. It is essentially a commuter campus, focusing on liberal arts education. Although an architectural masterpiece, the Chicago Circle campus still must make its mark academically. It has been subjected to the internal politics inevitable in a system as large as Illinois, which has an enrolment of nearly 60,000 students. For example, Chicago obviously offers a unique laboratory for urban planners and architects, but the stubborn Illinois faculty still retains the core of its urban planning-architectural faculty in the rural setting of Urbana, 150 miles to the south in a prairie atmosphere.

The university's medical school, however, appears to be more progressive. Its College of Medicine is the second largest in the United States, exceeded only by the University of Michigan. And, recently, the medical school has been developing branches in two other Illinois districts—Peoria and Rockford—to meet urgent health needs.

A problem that has for months disturbed the university's administration is the loss of federal government support for construction and operation of a major computer known as Illiac IV, which is now to be located at the Ames Laboratory in California. A building contracted for by the university as a home for Illiac IV in October 1969 and now nearing completion will be used, instead, by the Center for Advanced Computation, a research unit established at Urbana last summer. The Department of Defense changed its mind about locating the computer on the campus after Illinois, which had been relatively quiet, suddenly became the centre of student activism about two years ago. The computer, being built by Burroughs Corp at Paoli, Pa., pioneers a concept

originated by Dr Daniel L. Slotnick, professor of computer science at Illinois.

Student opposition developed after the campus newspaper, the *Daily Illini*, reported that the \$24 million computer would be owned by the DOD and was expected to create a body of scientific knowledge that will make possible larger and more powerful weapons and facilitate development of the Safeguard anti-ballistic missile. When the machine is plugged in, it was said, the world's computer capacity will increase 25 per cent. Defenders of the decision to move the site to California argue that the computer was so large and complex that the campus was not the best place to operate it and that costs would outrun original estimates to such an extent that the Defense Department might need help in financing it, say, from another governmental agency. Yet those close to the project say that the fact that the proposed Illiac IV building became a target of picketers had much to do with the decision in Washington to move a critical research tool away from a campus that might explode. Meanwhile, University of Illinois faculty members are working on basic research in computational techniques. Hopefully, their efforts will lead to more advanced systems, one of which could well be "Illiac V". But who will finance it and where will it be built if they develop such a complex tool? Those questions may well confront Dr Corbally in the not too distant future.

At an annual salary of \$50,000, Corbally comes to Illinois at a period when political leaders, fearing tax increases, are trying to pare the state's appropriations for higher education and demanding higher tuition rates for students who once could attend classes for almost nothing because it was a public institution. He thus faces demands for austerity on two fronts, the declining federal government support of research and development activities on the campuses, as well as pressures to reduce programmes and capital construction from local public funds.

Corbally comes to Illinois chiefly as an administrative technician. There is little to indicate that he was ever a great scholar. He earned a doctorate from the University of California at Berkeley with a major field in educational administration and finance. Before going to Syracuse as president, he was vice president for administration at Ohio State University in Columbus. Corbally is the author of a standard work on education administration. The fact that this type of a background intrigues a university search and screen community dramatizes that the boards of trustees, also frustrated by various problems, are turning to professional administrators rather than scholars with established reputations.

Short Notes**Interior Filled**

WILLIAM T. PECORA, director of the US Geological Survey since 1965, has been chosen as Under Secretary of the Interior. His selection is part of the restorative process following the recent decimation of the Department's top management. The White House's first choice for the post was James R. Schlesinger, assistant director of the Office of Management and Budget, who is looking for a new job reportedly because of being overruled on a matter of military budget cuts by presidential assistant Henry A. Kissinger. But western senators, who have a strong interest in the department, indicated that Schlesinger was too much of an easterner for their tastes.

Pecora's nomination is expected to pass Congress without difficulty, and he has also received the blessing of environmental lobbying groups such as the League of Conservation Voters and Friends of the Earth. Pecora joined the Geological Survey as a research scientist in 1939; his new job will improve his salary from \$36,000 to \$40,000 a year.

The post of Under Secretary of the Interior has been vacant since last February 19 when Fred J. Russell, a former Californian real estate dealer, was fired amid sighs of relief from mine safety reformers, conservationists, long-time officials of the department and a Congressman who described him as "a true troglodyte of the McKinley era". Russell's departure followed a purge of the department conducted by the White House around Thanksgiving day last year when the Secretary, Walter J. Hickel, and six of his aides were dismissed. The blacklist, apparently drawn up by Russell, included Dr Leslie Glasgow, a professor on leave from Louisiana State University, who was Assistant Secretary for Fish, Wildlife and Parks. "The Administration thought many decisions should be based first on politics and second on environment; I was just not political enough," Glasgow was quoted as saying a few weeks after his dismissal.

Breadlines into Ploughshares

A \$42 MILLION programme to help jobless aerospace scientists and engineers was announced by President Nixon on April 1 from the western White House at San Clemente, California. The chief purpose of the programme is to retrain these people in more socially relevant skills; it may also serve to undercut the \$500 million retraining programme for technologists which Senator Edward M. Kennedy has introduced into Congress. This new initiative is a more generous departure from the Administration's belief that unemployment will cure itself as the economy expands.

NEWS AND VIEWS

X-ray Astronomy's Big Step Forward

As everybody said it would, the first serious X-ray astronomy satellite has revolutionized the subject, if not overnight then in the short space of four months. Most astonishing is the discovery that the X-rays from the hitherto unsurprising source Cygnus X-1 are pulsed at a rate of probably about 15 pulses per second. Why Cygnus X-1 should have turned out to be the second X-ray pulsar when everybody had been turning their counters to the Vela pulsar is obviously going to be this year's talking point in astronomy. With murmurings about the possibility of Cygnus X-1 being a black hole, the discovery has naturally overshadowed the other achievements of the satellite, but since the launch in December it has doubled the number of known extragalactic X-ray sources from three to six, and altogether added thirteen new X-ray objects to the tally of almost fifty at the end of last year. All this was reported by Riccardo Giacconi of American Science and Engineering at the meeting of the American Astronomical Society in Baton Rouge, the first public report of the satellite data that had been widely anticipated after the successful operation of the satellite earlier this year.

Clearly, the success of the satellite—Explorer 42—is first of all going to be a valuable boost for the further launches which are planned in the Small Astronomy Satellite series, as well as being a timely vindication of the decision by the Space Science Board of the National Academy of Sciences to put their money on the series of High Energy Astronomical Observatories. But pulsar astronomers will be the first to be scratching their heads at the data on Cygnus X-1 which immediately turn upside down all the accumulated wisdom not only on the emission mechanism of pulsars but also on their place in stellar evolution. For to start with, Cygnus X-1 is by no stretch of the imagination a twin of the only other X-ray pulsar—NP 0532 in the Crab Nebula—or of the pulsar PSR 0833-45 apparently associated with the Vela supernova remnant and widely tipped as likely to be detectable in the X-ray band. To begin with, the most obvious difference is that Cygnus X-1 is not known as a radio source. The most pressing problem then will be to think of an emission mechanism compatible with the X-ray and radio observations, and clearly this is going to be a different problem to the Crab pulsar which generates strong radio pulses. Pulsar astronomers are now bound to be considering whether they have made a mistake in taking the Crab pulsar to be the archetypal pulsar.

Yet in the coming months Cygnus X-1 could turn out to be a refreshing new influence on pulsar theories, and, at first sight, it may be that the break in the spectrum of the Crab pulsar between radio and infrared frequencies—hinting at two different emission mechanisms which operate at low and at high frequencies—may be a clue to the link between the Crab and Cygnus pulsars. But how could the mechanism operating at the radio end of the spectrum be suppressed? In the optical part of the spectrum the knowledge that Cygnus X-1 is pulsing at probably 15 pulses per second (there still seems to be some uncertainty in the precise rate because of the influence on the data of the sampling rate) will help identify the source

among the background of Milky Way stars, and Jerome Kristian of the Hale Observatories is reported to be looking for optical pulses with the equipment that has been developed for searches for the optical counterpart of PSR 0833-45.

It also now seems that the use of the precisely known age of the Crab pulsar as a yardstick for measuring the ages of pulsars might have had a bad influence on theories of pulsar evolution. For the estimated age of Cygnus X-1 based on the repetition frequency is ten thousand years, yet there is no sign of the supernova remnant which should still be detectable around the pulsar. It could, of course, be invisible for some reason, but a more satisfying approach must be that the pulsar was not formed in a supernova explosion at all, although this is at odds with the direction that theories of pulsar evolution have been taking in the past year or so. It looks as though Cygnus X-1 passed through the stages of collapse without at one point being disrupted by a supernova explosion, although it seems premature to move immediately to theories involving a cloud of material in a vortex around a black hole to account for the X-ray emission.

If it had not been for Cygnus X-1, the Explorer 42 scientists would have been toasted for what they have added to knowledge of extragalactic X-ray sources. In addition to the radio galaxies M87 in Virgo and Centaurus A, and the quasar 3C 273 which were known to be X-ray emitters from rocket work and have now been confirmed by Explorer 42, the satellite has added the galaxy M84, also in Virgo, and, more exciting, two Seyfert galaxies, NGC 4151 and NGC 1275. The NGC 1275 result confirms data obtained from a rocket flight last year by Herbert Friedmann's group at the Naval Research Laboratory. That Seyfert galaxies should now be on the list is not altogether a surprise; indeed, astronomers would have been worried if they had turned out not to be detectable, because with their small, bright nuclei having optical spectra characterized by strong, severely Doppler-broadened emission lines, all the evidence suggests that Seyfert nuclei contain highly energetic processes. Presumably in the first instance the new discovery will be taken as further evidence that in some way Seyferts are intermediates between quasars and normal galaxies, although it is still not clear in which direction the evolution would proceed. Second, with these new data to hand, people will be discussing whether the total emission from Seyfert galaxies could possibly be the explanation of the unresolved X-ray background which is known to exist from rocket work.

At first sight Explorer 42 has also given a new view of the X-ray emission from the radio galaxy M87, for it now seems that the X-rays are coming from a wider area than that occupied by the optical galaxy. But the general distribution of the new X-ray sources that have been discovered, most of them presumably galactic, has not provided any surprises. There continues to be a strong concentration of sources near the galactic centre, where they are hard to identify optically among other material, with a smattering of sources elsewhere in the Milky Way.

Making or Rescuing Human Tumour Viruses?

BECAUSE the RNA tumour viruses are such notorious disrespecters of species barriers, more than one cautious tumour virologist has voiced the fear that growing an animal cancer virus in human cells may result in the creation of a human cancer virus. And on page 445 of this issue of *Nature* Aaronson reports experiments which go a long way towards fulfilling this prophecy. Last year in *Nature* (225, 459; 1970), Aaronson and Todaro reported the not surprising finding that eighteen strains of cultured human fibroblasts, all derived from one person, can be transformed, albeit inefficiently, by mouse sarcoma virus. They also found that the progeny viruses produced by their transformed human cells behaved like perfectly ordinary mouse sarcoma virus. In other words, the passage of the mouse virus in the human cells had not apparently caused any detectable change in the host range of the virus, which still transformed mouse cells far more efficiently than human cells. But on page 445, Aaronson tells quite a different story.

During the past months he has maintained some of the original transformed human cells which he and Todaro isolated last year. These transformed fibroblasts, as previously reported, initially released apparently normal mouse sarcoma and leukaemia virus. (Two viruses are involved because mouse sarcoma virus cannot be isolated free of mouse leukaemia virus, for the latter acts as a helper providing undefined but essential functions which the sarcoma virus genome cannot itself specify.) After sixteen weekly transfers, however, the "mouse" sarcoma virus liberated by these transformed human cells was found to have lost its ability to transform Swiss mouse cells but it now transformed human cells much more efficiently than before. Indeed, after such prolonged passage in human cells, the "mouse" sarcoma virus transformed human cells almost as efficiently as it transformed rat cells whereas before passage in human cells the virus transformed rat cells far more efficiently than human cells.

Clearly the host range of the virus had changed markedly as a result of its prolonged stay in human cells. This fact alone, of course, suggests that the changed host range results from some genetic change, rather than a phenotypic change, for example in the virus coat, which might be expected to be immediately apparent. This possibility has to be rigorously proven, however, for it has some startling implications. And so Aaronson passaged for three weeks the virus produced by the transformed human cells in rat cells, arguing that if the changed host range was phenotypic rather than genetic this passage might well eliminate it. But in fact after three weeks in rat cells the virus was still unable to transform mouse cells but transformed human cells even more efficiently. Aaronson next established that both the sarcoma and leukaemia viruses emerging from transformed human cells, after prolonged passage, had identically changed host ranges; both had apparently suffered the same genetic change. He then showed that the same type of sarcoma virus being helped by a different strain of mouse leukaemia virus suffered the same change after passage in human cells.

The two sorts of leukaemia virus helpers used have

distinct antigens so that they, and the sarcoma viruses they help, can be differentiated by immunochemical tests. Antisera directed against one sarcoma/leukaemia virus complex do not neutralize the other. As judged by this test the sarcoma/leukaemia viruses emerging from transformed human cells retain at least some of their murine antigens. The viruses adapted to human cells are still specifically inactivated by antisera against the corresponding parental stocks of mouse virus. On the other hand antisera against either type of adapted sarcoma/leukaemia virus complex inactivated both adapted virus complexes but had no detectable effect on either parental virus stock. The prolonged passage of different types of murine sarcoma/leukaemia virus complexes in human cells seems to result not only in a similar change in host range but also in the acquisition of common surface antigens that cannot be detected in the original stocks.

Accepting for the moment that these changes are genetic, how might they arise? There are two obvious possibilities; multiplication and selection of mutants arising during the passage in human cells or recombination between human cell and mouse viral genomes, and Aaronson plumps for the second. All his results certainly suggest that the change in host range of these adapted viruses is genetically stable and, because two antigenically distinct types of sarcoma/leukaemia virus complex are identically changed, it seems perhaps unlikely that this change would arise from the selection of random mutations in independently growing populations. Furthermore, of course, the discovery of reverse transcriptase provides the enzymatic machinery necessary for recombination between the genome of a tumour virus and its host. There is now little doubt that transformation by the RNA tumour viruses involves the synthesis of a double stranded DNA copy of the transforming viral genome, and the integration of this viral DNA into one or more host chromosomes. Although the mechanism of this process remains obscure it seems likely that it involves breakage and rejoining of DNA molecules, events which probably also give rise to recombination. One does not therefore have to stretch the imagination far to picture recombination occurring between a tumour virus genome and that of its host.

What sort of genes are likely to be involved in this process? As Aaronson speculates, the recombination might be between the mouse sarcoma virus genomes and those of putative, latent human viruses, integrated into human chromosomes. If this were the case the new antigens on the surface of recombinant, adapted mouse sarcoma virus might be specified by genes of the putative, latent human virus. In other words these antigens might provide markers for human cancer viruses. Needless to say, all this is for the moment pure, and some may say unwarranted, speculation. But it should not be all that difficult to put it to the acid test of experiment and in any event the changes in mouse sarcoma virus after prolonged passage in human cells raise some fascinating questions about the stability and determination of the host range of animal tumour viruses. Aaronson may not have rescued human tumour virus genes but he has gone some way along the road to making such a virus.

PROTEIN STRUCTURE

Darwin among Enzymes

from our Molecular Biology Correspondent

X-RAY crystallography is a many-splendoured science, which is now being enlisted in the service of molecular evolution. The evolutionary divergence of protein molecules has been studied primarily in terms of primary structure. The biggest effort has been mounted on cytochrome *c*, which being small and a constituent of the most rudimentary as well as the most advanced forms of life, can scarcely be bettered for the purpose. The cytochrome *c*s of a great variety of species, from the kangaroo to the castor bean, have been sequenced, to the point that the irreducible core of invariant features, which are presumed to be indispensable for biological function, can now be defined with a fair degree of confidence. So far, thirty-five of the hundred odd amino-acids remain invariant, including one run of eleven, and almost as many again are subject only to conservative substitutions. In all cases there is a high lysine content, and the protein is very basic. The basic, acidic and hydrophobic residues are strikingly clustered, and glycines, hydroxylic and aromatic side chains, and the haem-linked groups are strongly conserved.

Dickerson *et al.* (*J. Biol. Chem.*, **246**, 1511; 1971), with the aid of 2.8 Å resolution structures of ferricytochrome *c* from two sources—horse heart and bonito—have now set out to throw light on the path of evolution in terms of the stereochemistry. The molecule gives the appearance of being folded around the haem group, which is lodged in a crevice, attached on one side to two cysteines and a histidine, on the other to methionine (thus resolving a venerable argument concerning the nature of the sixth haem ligand). One of the propionic acid side chains of the haem group is secured in the interior of the molecule by a criss-cross of hydrogen bonds. Across the crevice is the only α -helical segment of the chain, and as Dickerson *et al.* note, the various rules purporting to relate helicity to sequence fail prodigiously when applied to cytochrome *c*. Bends in the chain, in the form of 3_{10} -helices are present, and are now beginning to appear as one of the generalities of protein structure. Cytochrome *c* has the form of a coating of predominantly polar side chains over a shell of hydrophobic side chains on the inside ("oil drop"), a large number of the latter packing closely round the haem. The invariant residues are distributed as follows: besides the four linked to the haem group, the tract 70–80 constitutes one side of the haem crevice (containing the methionine ligand); three invariant aromatic resi-

dues reside in a channel to one side of the haem, which, it is surmised, gives access to the surface for electron transfer; two invariant prolines are found to one side; a channel on the opposite side contains two aromatic side chains. Lysines are clustered round the outlets of these channels, and probably represent an operational binding site. On the other side of the molecule from the haem crevice is a cluster of nine acidic groups, which are surmised to be implicated in binding functions. Further generalizations that emerge from inspection are that prolines and glycines, which tend to be invariant, exert geometric control over bends in the chain, and the hydroxylic side chains are involved in internal hydrogen bonds. The highly variable positions occur at outside corners, where the side chain can float freely in the solvent.

Two related proteins, widely separated in evolutionary terms, are mammalian myoglobin and the monomeric insect haemoglobin, erythrocyruorin. The structure of the latter was determined by Huber and his colleagues, who now survey the differences and similarities between the two molecules (Huber *et al.*, *Europ. J. Biochem.*, **19**, 42; 1971). The structures are clearly broadly similar, though only some 20 per cent of the residues are conserved. The situation is somewhat complicated by some incompatibilities between the structure and the published sequence, some parts of which Huber *et al.* have tentatively revised. Relative to the myoglobin chain, that of erythrocyruorin shows several deletions, a phenomenon which has considerable structural consequen-

ces, in particular a bodily displacement of 2 Å of one of the long helices (B helix). There are other displacements and distortions of α -helical segments, but the most striking feature of the comparison undoubtedly concerns the environment of the haem group. Only five of the eighteen residues packed round the haem are conserved. The functionally important C-terminal tyrosine, common to all mammalian haemoglobins, is replaced by erythrocyruorin by a methionine. A large number of phenylalanines occupy the interior, no less than seven in the haem pocket, and a glutamic acid replaces the distal histidine, long supposed indispensable for oxygenation (though recently found to be replaced by arginine in an abnormal human haemoglobin variant).

Robertus, Alden and Kraut (*Biochem. Biophys. Res. Commun.*, **42**, 334; 1971) have used X-ray crystallography to settle a long-standing conjecture that subtilisin BPN' ('Nagarse'), as produced in Japan, is the self-same enzyme as subtilisin B, or 'Novo', from Denmark. Peptide mapping reveals no differences, but cannot exclude some conservative substitutions, and the enzymatic characteristics are also indistinguishable. The nature of the parent organisms is veiled in commercial secrecy, for they are used in the manufacture of enzyme detergents. The 2.5 Å difference map of Robertus *et al.* settles the issue; it is essentially featureless, but for small aberrations, thought to be differences in bound solvent distribution, resulting from differences in crystallization conditions. Evidently the products will wash equally white.

A New View of Cosmic Infrared Sources

COSMIC infrared sources are usually explained by shells of dust grains around ultraviolet sources, the dust converting the energy to infrared wavelengths. An alternative view is put forward in next Monday's *Nature Physical Science* in which N. C. Wickramasinghe (Institute of Theoretical Astronomy, Cambridge) suggests that the absorption of cosmic ray energy rather than ultraviolet energy by dust grains, and its re-emission in the infrared, can better account for the observational facts. What has inspired Wickramasinghe to develop this viewpoint is that the short term variability of cosmic infrared sources indicates that they have dimensions of the order of a parsec or less, yet using conventional dust models and ultraviolet sources it is hard to see how the dimensions could be less than ten to a hundred parsec.

Wickramasinghe has therefore considered in detail what happens when a cosmic ray nucleon traverses a dust grain, and points out that cosmic ray

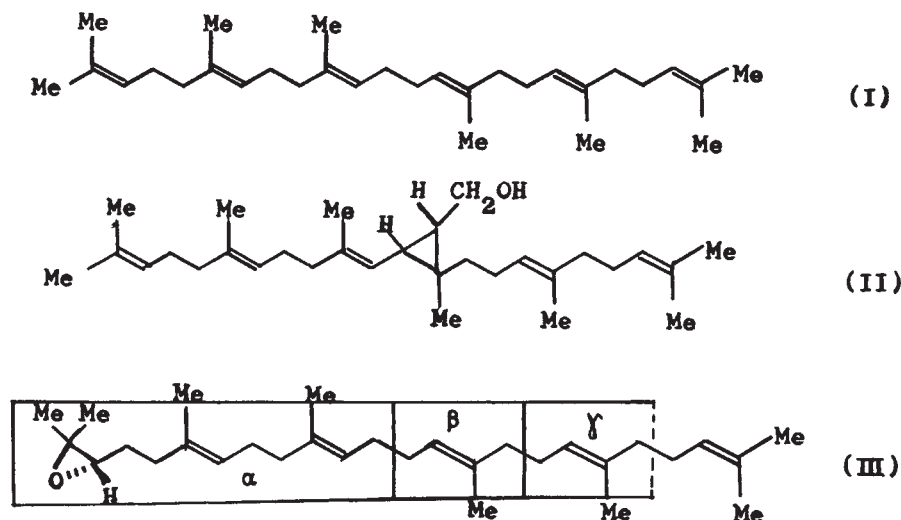
heating could be the dominant source of grain energy near objects such as supernovae shells or other exploding objects. He shows that intensity variations on time scales of days or months, as have been observed, are compatible with this model, and the amounts of dust required seem reasonable. In other words, the infrared emission can be generated in sources that are an order of magnitude smaller than would be required if ultraviolet photons were the energy source.

Wickramasinghe has also been able to bring into his account other features of the infrared emission from galaxies, notably a turnover in the spectrum which occurs at 2.2 μm which he explains in terms of the effect on the grains of sputtering. It will be interesting to see whether the conventional explanations of the infrared sources can be improved to deal with the difficulty highlighted by Wickramasinghe's article, or whether his idea will come out on top.

STEROLS

Closing and Opening Triangles

from our Biological Chemistry Correspondent



CORNFORTH and Popják's classical studies of the route by which mevalonic acid is converted into squalene and thence into sterols provided stereochemical detail which was little short of amazing at the time but left unsolved the problem of just how two C-15 farnesyl residues are united, tail-to-tail, to give squalene (I). The enzyme responsible, squalene synthetase, proved difficult to manipulate, but two research groups eventually culled from its grasp similar, possibly identical, presqualene alcohols in the form of pyrophosphate esters. Provisional structures were proposed by Rilling and by Popják for their C-30 intermediates though Rilling's failed to survive the test of a Corey synthesis and Popják's awaits such trial.

Rilling thereupon revised the structure of his cyclopropanecarbinol to (II) and Crombie has now achieved a neat synthesis of this material (*J. Chem. Soc. D.*, 218; 1971). Although the methods used gave all four diastereoisomeric racemates, these proved separable by the combination of gas-liquid and thin-layer chromatography. Just one of the mixtures of enantiomers had spectra identical with Rilling's precursor and could be converted into squalene by a yeast microsomal preparation with fair efficiency.

If (II) is really a true intermediate in the biosynthesis of squalene, the formation and subsequent destruction of a cyclopropane ring must surely be one of nature's most devious methods for making a single carbon-carbon bond.

In contrast, recent progress in van Tamelen's group at Stanford University (*J. Amer. Chem. Soc.*, **92**, 7202, 7204, 7206; 1970) shows that the further

enzymatic process which converts squalene 2,3-oxide (III) into sterols exhibits impeccable chemical behaviour. Both chemical and enzymatic cyclizations of many analogues and derivatives of (III) have reduced the features essential for enzymatic cyclization to the epoxide-tetra- π -bond system (III, α , β , γ). They also suggest that much of the steric control of structure developed in the product sterol rests, as predicted, solidly on the chemical foundations of methyl-hydrogen migrations.

Hope of Safer Human Marrow Grafts

A NOVEL technique which may eventually prove of value in the development of human marrow grafting is reported in next Wednesday's *Nature New Biology*. L. J. Cole and S. E. Maki of the Stanford Research Institute, California, have devised a way to overcome the chief obstacle to the widespread use of bone marrow grafting in the treatment of leukaemia and immunological deficiency diseases. This obstacle is the development of graft versus host (GVH) disease which swiftly follows transplantation, and which is the result of the immunological reaction between the cells of the donor graft and the tissue antigens of the host.

Cole and Maki have inactivated the cells in the donor marrow which are responsible for initiating the lethal GVH response, by treating them with heterologous anti-mouse γ -globulin serum. The efficacy of this method hinges on the fact that the immunologically active marrow donor cells—the lymphocytes—carry on their surface immunoglobulin-

ORBITALS

In Honour of Coulson

from a Correspondent

PROFESSOR CHARLES COULSON'S sixtieth birthday conference in Oxford on April 1 and 2 provided his many friends and pupils with a very welcome opportunity to express their affection and gratitude. The conference was generally concerned with orbitals in atoms, molecules and crystals, as was very proper to honour the founder of quantum chemistry, but covered a wider field, from density matrices to the properties of the analogues of vitamin B_{12} (Dr M. Green, University of York).

One session of particular interest concerned the possible killing off of orbitals, to be replaced by density matrices. Professor E. Bright Wilson (Harvard University) pointed out the difficulties caused by the fact that the second order density matrix must obey an extraordinarily large number of necessary conditions, and he was pessimistic over the chances of ever constructing it. Professor A. J. Coleman (Queen's University, Ontario) brought a ray of hope, based on abstract set theory, but Professor Coulson and Professor N. H. March (University of Sheffield) were more optimistic and suggested that perhaps all the conditions will turn out to be satisfied automatically, or may not be so important. Related to this was the lecture by Professor March who described his group's important work towards the solution of the many-body problem.

like molecules which can combine with antigen in which the active sites are of complementary configuration. Coles and Maki therefore treated mouse antigen-reactive lymphocytes with either normal rabbit serum (NRS) or anti-mouse 7S γ -globulin serum. Theoretically, the anti-mouse serum should bind to the reactive sites on the surface of the lymphocytes so rendering them immunologically inactive, and indeed Coles and Maki found that sub-lethally irradiated mice injected with the anti-mouse serum neither died nor showed signs of runting disease. In contrast, all the mice injected with NRS showed signs of runting and eventually died. Further experiments in which allogeneic bone marrow cells were treated with the heterologous anti-mouse serum were equally encouraging, especially because the serum does not seem to interfere with the ability of bone marrow stem cells to form haemopoietic colonies, a critical feature in the repopulation of haemopoietic tissues.

In the field of molecular theory, Professor R. G. Parr (Johns Hopkins University) presented some novel relations between the vibrational force constant k of diatomic molecules and the atomic numbers Z_a , Z_b of the component atoms. These relations, of the type $\ln(k/Z_a) = AR + B$ (A , B constants, R = equilibrium interatomic distance) reflect the exponential decay of the radial wavefunctions. Other contributions carried previous calculations to higher approximations, for instance Dr C. W. Haigh (University College of Swansea) and Dr R. B. Mallion (University of Oxford) on the perturbation of a π -electron cloud by a magnetic field, or Dr M. Thomas (University of Oxford) on the explanation of why the apparent bond length measured by X-rays is less than measured by electron or neutron diffraction.

One of the most interesting sessions was that on the hydrogen bond. The problem of its nature has not yet been fully solved. A discussion of the various contributions to the H-bond energy was presented by Dr L. C. Allen (Princeton University) and Dr P. A. Kollman (University of Cambridge): a molecular orbitals calculation could predict, at least qualitatively, the energy of the bond in H_2O -HF dimers, though the value was very sensitive to the choice of the basis set. Professor M. Kasha (Florida State University) succeeded in unravelling the complicated phenomena (charge transfer, simultaneous transitions, successive excitations, and the like) which occur when molecular oxygen absorbs radiation.

There were also contributions on scattering theory, band theory, polarons, algebra on computers. It was altogether a most enjoyable meeting, demonstrating clearly, as remarked by Professor J. H. van der Waals (Leiden University), the stout trees that have grown from the seeds sown by Coulson perhaps twenty years ago.

SINGLE CELL PROTEIN

Doing the Sums

from our Microbiology Correspondent

PROTEIN shortages of 10 and 22 million tons are predicted by the Food and Agriculture Organization by the years 1980 and 2000 even if all present resources are fully exploited. Such statistics have induced a spectacular response from biologists who see microbiologically produced protein—the now familiar single-cell protein (SCP)—as the prime hope for making good this deficit. The big attraction of SCP is the possibility of utilizing cheap raw materials as fermentation substrates and these include hydrocarbons, molasses, whey, sulphite waste liquor and other wood products. A major

uncertainty surrounding the development of SCP, however, is one of economic feasibility; the base line against which to make an economic assessment is about ten cents per pound for cotton seed, soy and peanut proteins. The appraisal of microbial protein by Vilenchich and Akhtar (*Process Biochem.*, 6, 41; 1971) is therefore timely and should help to orientate both academic and commercial groups interested in this project.

SCP has several advantages over traditional animal and plant proteins; the higher nutritional status and very much shorter time required to double the mass of the product are two of the most important. Offsetting these advantages is the question of market acceptability, especially when a product for direct human consumption is considered. The cost of processing SCP

for human diets is high and the final product is likely to be three to four times more expensive than plant proteins. But when petroleum is used as the raw material little processing is required to produce animal feed supplement and the cost can be as little as six cents per pound.

Vilenchich and Akhtar point out that single-cell protein research has centred so far on three basic problems—searching for the cheapest suitable fermentation substrate, searching for organisms which, in addition to possessing the right nutritive properties, grow rapidly and produce the highest yields on the chosen substrate and solving bioengineering problems encountered during industrial scale production. Scientific opinion, including that with a commercial interest, favours hydrocarbon fermentation as that offering the

Enzymes for Transformation

RNA tumour virus particles carry into the cells they infect all the enzymatic machinery necessary for the malignant transformation of their hosts—that is the fascinating conclusion Howard Temin and his colleagues have reached from their latest investigations of the range of enzyme activities present in Rous sarcoma virus particles (see next Wednesday's *Nature New Biology*). The epoch making discovery of reverse transcriptase in RNA tumour viruses, independently reported last June by Temin's group and Baltimore, explained how these viruses can stably transform cells. For reverse transcriptase can use the single stranded RNA genome of these viruses as a template for the synthesis of, first, a complementary DNA strand and then a double helical DNA. And this molecule, containing all the genes of the infecting virus including those responsible for transformation, can then be integrated into the chromosomal DNA of the infected cell and inherited by each daughter cell at mitosis.

The integration of tumour virus DNA into a host cell chromosome could conceivably be brought about by enzymes existing in the cell before it is infected. The enzymes involved in recombination, for example, could, in theory at least, do the job. But it seems, from the experiments of Mizutani, Kodama, Wells and Temin, that in fact the virus carries with it its own integration machinery. They have found that in addition to reverse transcriptase Rous sarcoma virus particles contain a DNA endonuclease activity which can cut long DNA chains into shorter pieces, and a DNA exonuclease activity which can digest DNA by clipping nucleotides one by one from the end of a chain. Furthermore, these viruses contain a

DNA ligase activity; that is to say, they have an enzyme which can join together the free ends of two DNA chains.

Although they have yet to prove that all these enzymes are involved in the process of transformation their very presence in the virus particles suggests the following sequence of events. After infection, reverse transcriptase makes a double stranded viral DNA. The two nucleases then cut some part of the DNA of a host chromosome and trim away a gap. The viral DNA is then inserted in the gap and sealed into the host DNA molecule by phosphodiester bonds formed by the DNA ligase.

Most cells transformed by an RNA tumour virus continue to produce progeny virus particles which are budded from the surface of the cell. No doubt the integrated viral DNA molecules act as template for the transcription of viral RNA which can then be wrapped up into progeny particles; but until the advent of reverse transcriptase attempts to detect these progeny RNA molecules in the nucleus of cytoplasm of transformed cells were not crowned with great success. With reverse transcriptase, however, Green and his colleagues have been able to make themselves a highly specific probe for the missing RNA. As they report in Wednesday's *Nature New Biology*, radioactive murine sarcoma virus DNA, made with reverse transcriptase, hybridizes with viral RNA present in transformed cells. They estimate that as much as 5 per cent of the RNA in the nuclei of cells transformed by mouse sarcoma virus is virus specific and that 0.5 to 1.0 per cent of the RNA in the cytoplasm is viral RNA. So another link in the story of RNA tumour virus replication has been found.

best prospects for industrial production of microbial protein. The returns from gaseous hydrocarbon fermentations are compromised at present, because the very much cheaper cost of the substrate is laid against less favourable growth and nutritive characteristics. Nevertheless, both engineering and microbiological technologies can be expected to improve this position.

Apart from hydrocarbons, various plant materials have attracted attention in the contexts of SCP and sugar production. Thus, numerous schemes, many of them patented, have been suggested for the microbial utilization of wood, sulphite waste liquor, bagasse and related materials. Fodder yeast production on sulphite waste liquor is one of the few successful processes of this type to emerge; and the attempts to produce glucose syrups from cellulose have been discussed previously in this column. The development of an SCP process based on wood hydrolysis products has also received attention and Russian interests in this field have been made known recently (*Nature New Biology*, **230**, 100; 1971). Kobayashi's review (*Process Biochem.*, **6**, 19; 1971) of chemical wood hydrolysis and the utilization of wood sugars for fermentation makes interesting reading for those who see this process as a cheap source of cellulose.

of fast reactors neutron irradiation to high doses at elevated temperatures was almost impossible. The consequences of such swelling must be taken into account in the design and operation of fast reactors; for, as a result of non-uniform damage rates throughout a reactor, some key components are expected to suffer distortion as a consequence of differential swelling. Such distortions must be minimized by operational or engineering modifications which may carry economic penalties, or alternatively by using different materials which exhibit greater resistance to swelling.

The first major conference on void swelling was organized by the British Nuclear Energy Society at the University of Reading on March 24 and 25. The conference was opened by a review of neutron induced voidage by Drs K. Q. Bagley, J. I. Brammon and C. Cawthorne (UKAEA, Dounreay) which presented some of the latest results and proved most valuable. Much of the current experimental work on void formation has been carried out using charged particles from accelerators or by electron irradiation in the high voltage electron microscope. Such techniques have been able to simulate the void swelling that occurs after many years irradiation in reactors in

times of less than a day and for this reason two complete sessions at the meeting were devoted to these studies; reviews were presented by Drs R. S. Nelson, D. J. Mazey and J. A. Hudson (AERE, Harwell) and Dr D. I. R. Norris (CEGB Berkeley Nuclear Laboratories).

The principal results to emerge from the meeting were that materials such as well annealed nickel, copper and stainless steel all show significant void formation. For instance, swellings up to about 10 per cent were predicted to occur in commercial fast reactor components after irradiation times equivalent to the life dose of the fuel, but if these same materials are previously cold worked they show reduced swellings. Perhaps the most significant development from both reactor and accelerator studies is that alloys such as Nimonic PE16 exhibit dramatic resistance to swelling. For instance, after irradiation to high doses at 525° C the swelling is more than an order of magnitude smaller than for steel. This result is thought to be a consequence of the very fine precipitates within PE16 which cause recombination of the displaced atoms, together with the fact that such precipitates act to pin the radiation induced dislocation network on a very fine scale.

NUCLEAR REACTORS

Void Swellings

from a Correspondent

THE fact that atoms are displaced from their normal sites within a solid during irradiation with fast neutrons from nuclear reactors is well known and has provided a fruitful field of research since the early 1940s. It was not until 1966, however, that C. Cawthorne and I. Fulton at the UK Atomic Energy Authority's fast reactor station at Dounreay discovered a new radiation damage phenomenon, namely "void formation".

Careful electron microscopical studies of fast reactor fuel cladding from the Dounreay Fast Reactor (DFR) revealed a high density of small cavities with diameters of 100 Å; such cavities were shown to be essentially empty and were therefore called voids. The creation of such cavities within a solid implies that the external dimensions of the solid must increase in order that the total quantity of solid material stays constant. It was soon found that certain reactor components had indeed increased their external dimensions and consequently suffered a density decrease.

The phenomenon of void swelling, as it has come to be known, was not expected, chiefly because before the advent

Synthesis of SO₂ Metal Ion Clusters

THE pioneers of the study of ions in the gas phase, such as Thomson and Aston in 1910 to 1920, expected the charged species to be very similar in type to known neutral species, from which they would be more or less directly formed. Although they had one or two surprises which were contrary to this expectation, they would surely be amazed at the development of the investigations in just over half a century since their own work was carried out. This is strikingly underlined by an account of work by Tang, Munkelwitz and Castleman in next Monday's *Nature Physical Science*.

Tang and his colleagues at Brookhaven National Laboratory investigated the results of introducing gaseous Na⁺ ions into a chamber filled to a pressure of 4 torr with SO₂. Ionic species extracted from the other end of the chamber showed stable combinations of up to five molecules of SO₂ into the ion. A similar effect with H₂O was confirmed, and a mixture of the two vapours was found to yield all possible combinations of the two adducts around the Na⁺. Further work, to be described fully later, showed similar effects when NO⁺ was substituted for Na⁺. A great deal of work will evidently be necessary before the full story of the structure, relative

stability and rates of formation of these species has been told.

The interest of such an extension of knowledge of ion chemistry reaches out in many directions. It is expected to have a bearing on the study of co-ordination complexes in liquid and solid phases. Studies of mobilities of ions in gas and liquid phase alike will have to take note of such effects, providing links with electrochemistry and with the study and varying uses of electric discharges. A very important and obvious similarity to the conditions of the ionosphere and upper atmosphere is pointed out by the authors, and this may indeed be a source of inspiration for the work. Studies of possible ion-molecule reactions are equally relevant, however, to the high pressure environment of a gas-cooled atomic reactor. A further branch of interest may lie in the study of the atmosphere which we breathe.

As much as a decade ago, Westermarck published (*Nature*, **189**, 910; 1961) observations of the ionic content of the atmosphere caused by internal combustion energies and other common devices. Further such studies might show a population of ions of most surprising constitution and stability, perhaps tending towards the characteristics of an aerosol.

Likelihood of Human Pheromones

A. COMFORT

Department of Zoology, University College, Gower Street, London WC1

A direct search for functional human odours might yield some very valuable results.

THE likelihood that there are functional human pheromones has been both asserted¹⁻⁴ and denied⁵, both without direct experimental evidence: the finding of clear pheromonal effects in monkeys^{6,7} and the recent observation of menstrual synchronization between close friends⁸ reopen the possibility more definitely, and make direct experiment obligatory.

The practical importance of such research lies in the possibility of primer control over human endocrine cycles and reproduction generally: if this exists, it might open a new chapter in reproductive pharmacology at a time when it is badly needed. Compared with drugs, pheromones are strikingly economical in quantity, many operating at a level of molecules rather than milligrams. Even the study of simple "releaser" effects could clarify a field of human, and especially developmental, biology which has been so far suspected rather than elucidated. Odour fingerprinting techniques and gas chromatography⁹ now make the detection and preparation of human pheromonal agents feasible if they exist.

Developmental Effects

Sexual releaser effects of odours in man have been recognized throughout human experience, even in cultures which found the idea embarrassing, and the richness of human olfactosexual behaviour was fully documented by Havelock Ellis¹⁰. All releaser effects in man tend to be more variable than in lower mammals because of the large variety of human signal systems and the size of the override from learned or conditioned behaviour, which is such that not even the human sex object is irrevocably fixed. The large observed individual variation in conscious olfactory awareness is almost certainly in part genetic, but psycho-analytic writers have both suggested and documented the possibility of a special role for odour in infant psychosexual development¹¹⁻¹⁵. According to this view, attraction to the odour of the opposite-sex parent and avoidance as a threat of the odour of the same-sex parent act as biological triggers for the Oedipal responses, an idea of much biological interest. This would represent a case intermediate between releaser and primer conditions, and possibly a programmed "temporary organ". Groddeck¹⁶ argued that man is as macrosmatic as the dog, but represses the capacity in adult life for psychosexual reasons. Without accepting this view, it is still credible that some part of human olfactosexual response may be confined to infancy and childhood, and subsequently "turned off", or altered in direction, either by a process of repression or, as in other mammals, by the advent of adult sex hormone status, most adult pheromone response being either androgen or oestrogen-dependent. The complexity of human psychosexual development is likely to produce unique effects on adult response not seen in other mammals.

Adult Effects

Adult odour releaser effects in man as embodied in sexual behaviour are attractant, ancillary to more familiar signal systems, and often overridden by individual experience, though still serving to synchronize intercourse with ovulation: beside male-female attraction, or bonding during pregnancy, they may possibly, on the mammalian model, include adult male-male dominance or hostility. Wider odour communication of states of mind, as postulated by Wiener², is ill-confirmed in other mammals and difficult to separate from other human subliminal cues. The value of seeking for the more tangible and important primer effects, which have not so far been suspected, depends on the following principal arguments.

(1) Pheromonal primer effects are near-universal in social mammals, including primates.

(2) Releaser pheromone effects exist in man, at least in larval forms, and some involve pheromones of other mammals (musk, civetone). These in nature are rarely simple releasers, but combine priming and other effects—thus one male odour may serve to mark territory, assert dominance, repel rivals, attract females and synchronize their cycles. Unless man is a wholly special case, or his use of this potential response system has been taken over by anticipation, as part of infantile rather than adult psychosexual mechanics, then similar effects are to be looked for in man also.

(3) In mammals as against insects, functional and species specificity do not seem to depend on a multiplicity of special substances. Cross-specific reactions are common (humans react to musk; bulls, goats and monkeys to the odour of women): interspecific bars between near species (dog/fox) are probably effected by addition or by concentration, the basic effector molecules being widespread. The physiological effect of similar molecules is likely to be similar between man and other mammals in which priming is known to occur.

(4) Humans have a complete set of organs which are traditionally described as non-functional, but which, if seen in any other mammal, would be recognized as part of a pheromone system. These include apocrine glands associated with conspicuous hair tufts, some of which do not produce sweat and must presumably produce some other functioning secretion¹⁷; a developed prepuce and labia, and the production of smegma. This system in adults seems over-elaborate for the relatively small releaser role of odour in most cultures. The amputatory assault on these recognizable pheromone-mediating structures in many human societies implies an intuitive awareness that their sexual function goes beyond the decorative. A conspicuous and apparently unused antenna array presupposes an unsuspected communications system.

Patterns of Response

The nature and function of such possible communications can be inferred from mammalian models, to indicate the types of priming which might be expected in humans. It is known that women have the greater olfactory sensitivity to most mammal odours, that this is oestrogen-dependent and, in the case of exaltolide, cyclical^{19,20}. Thus women detect, and react to, boar taint in pork far more readily than men²¹, the substance detected being apparently 5 α androst-16-en-3-one²².

A similar material occurs in human male urine, and in female urine during the luteal phase²³.

Since human male sexual behaviour is non-cyclical and not dependent on female receptivity, the female > male influence may well be releaser only, except possibly in infancy, or in accelerating puberty, and relatively nonspecific. It is not clear why odour release should be enhanced at the infertile time of menstruation, unless it overrules an infantile anxiety. The most likely true primer effects would be female > female or male > female—McClintock's chief example, if it is pheromonal, would be of the first kind: in this event male > female effects could also be sought with virtual certainty. The most likely of these, judging from mammalian form, are cycle modification or initiation²⁴, seen in mice, most herding animals (sheep, pigs) and, among primates, in lemurs²⁵; and acceleration of puberty²⁶. Human puberty certainly regressed to a late age during the height of Victorian purdah, and has since got steadily earlier²⁷: this has occurred in both sexes and may well involve social factors—a pheromonal effect would be impossible to isolate. If it existed, it must presumably, in view of human family structure, depend on reinforcement by strangeness, and the presence of nonfamilial individuals. A pheromone effect triggered only during sex play or coitus could not easily be separated from the effects of direct stimulation, though there are such pheromones in primates^{6,7}, and human sex play has a large, though tabooed, orogenital component. Natural pheromone effects on fertility, implantation and the like²⁸ seem more remote in man, though they might be produced by synthetics and would be of great importance if found. The conceptuant and abortifacient effects of odour figured in mediaeval medical folklore, and musk and civet were among substances so credited.

A male > male effect cannot be ruled out. Its most likely form, on mammalian analogy, would be the release of aggression or submission, but distaste for foreign male odours seems to be reversible, for example in homosexuals¹⁰, and the fact that human male bonding is prominent could suggest that other interactions, such as puberty-timing in the male group, might be expected. The work of Kalogerakis¹⁴ implies a dominance effect between mature and immature males.

Chemical Substances

Beside the collection of considerable knowledge of insect pheromones, little has been done on mammalian smell components. The known candidates for pheromonal roles are those "self-selected" by man and used in perfumery (muskone, civetone, castoreum, and synthetics such as exaltolide), those derived from steroids and observed incidentally, such as boar taint, and a few special cases (*cis*-4-hydroxydeca-6-enoic acid lactone in deer tarsal gland odour^{29,30}; response of some cat strains to valerianic acid and nepetalactone³¹).

The substances of initial choice as probable releasers and possible primers in man are all musk odours (steroids, large-ring cycloketones and lactones)³². The part played by 6, 8, and 10-carbon acids and lactones is unknown, but like the accessory non-steroid components of sweat, of smegma and of boar odour^{22,33,34} they probably have to do with detailed specificity. On this model the pheromone molecule is the key, and the subsidiary "notes", the wards adapting it to a particular biological lock, and possibly needed for reinforcement. Application of pheromones would probably require us to take account of both systems—the degree of functional specificity is likely to be as high in mammals as in insects, but more complex, involving, for example, individual recognition. To this end the odour fingerprint approach of Dravnieks seems more promising than classical chemistry. Known musks might well serve as initial markers: the musky odour of human urine appears to be due to the -3-ol precursor of boar taint³⁵ and nearly all 5 α and 5 β androsthenones, as well as progesterone, have musky odours³⁶. The identity of mouse primer pheromones could be simply attacked by the methods of concentra-

tion used with insects—so far no article on these lines has appeared.

Sources of Pheromones

Many mammalian pheromones seem to be urinary, though specialized secretions are also common. Contact with urine plays little part in human relations, though it may be emphasized in paraphilias. More likely vehicles in man are the skin, including axillary and pubic apocrine glands and hair tufts, which resemble the deer's tarsal organ, and the smegma. The function of this secretion has been little studied, apart from its possible role in carcinogenesis. In the boar, its function seems to be to acquire odorous substances, either by secretion or fixation from preputial-sac urine, and "hold" them, possibly for conversion to an active state by bacterial action. Excision of the preputial glands reduces boar taint³⁷ although the source of the steroid precursor is the gonad and adrenal³². Humans lack the preputial sac, but male smegma contains a number of fixatives, including squalene³⁸ and β -cholesterol esters, as well as other uncharacterized steroids³⁹. Some odorants may be directly secreted, others fixed from urine. Odorous drugs, for example phenylethylhydrazine, are rapidly detectable in the human male genital odour. Deer musk is a direct preputial gland secretion and a fixative for secondary odours. Apocrine glands contribute to total body odour, but a smegma pheromone would be exposed precoitally with exposure of the glans, and would thus be analogous to the "response" substance in male moths; that is, a direct stimulus to receptivity. Odours fixed from a partner might also have a "playback" function, as in offspring labelling. Human female genital odour components have been studied by gas chromatography, but only in relation to the elimination of bacterial odours⁴⁰.

The axillary secretion is a far more likely source of human social pheromones—possibly specialized, in view of the erect posture of man^{15,19}. Odorous steroids such as progesterone are rapidly transferred to objects handled by a pregnant woman through the sweat generally, and the large human apocrine glands may be centres of such a function. The hair tufts probably serve as odour diffusers, as in deer, and may harbour activating bacteria⁴¹. Many substances are probably involved, including long-chain acids and lactones. Caproic, caprylic and capric acids were named from their "hircine" odour—human sweat may also smell of coumarone. Research in this field has been largely limited to deodorants and to identification of mosquito attractants in sweat^{42,43}. The peculiar odour of schizophrenics' sweat has been traced to *trans*-3-methyl hexanoic acid⁴⁴—its significance is unknown.

Model for Effects

A model of possible human pheromone effects can be plausibly constructed. The responsible substances are likely to occur in apocrine sweat and in smegma. They are likely to include odorous steroids, large-ring ketones, or other substances perceived as musk-like. Accessory odours probably determine behavioural specificity rather than direct physiological action. Primer effects are most likely to be seen in the female; female > male effects may be limited to attraction, erection and so on and male > male effects may include dominance and attraction (bonding).

Odours fixed from a receptive partner may serve a "playback" function. Reinforcement by strangeness may occur. There may be an extensive biology of imprinting and so on by odour in infant-parent and child-parent relations, which later undergoes repression or modification. Releaser and primer effects probably depend on the same materials, and may include substances fixed from urine, semen, or the sexual secretions of the other sex by smegma. The existence of primer effects in humans is unproven but likely. Odour fingerprinting and gas chromatography render the testing of these hypotheses immediately practicable.

For references, see p. 479.

Synchrotron Radiation as a Source for X-ray Diffraction

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Some preliminary results have been obtained with synchrotron radiation from the 7.5 GeV electron synchrotron Deutsches Elektronen - Synchrotron (DESY) in Hamburg as a source for X-ray diffraction.

WHEN an electron is accelerated it emits radiation. At the very high energies used in DESY, the emitted radiation is confined to a narrow cone about the instantaneous direction of motion of the electron. Thus the synchrotron radiates tangentially. Synchrotron radiation is polychromatic, with a peak in the X-ray region for an electron energy of 7.5 GeV (see ref. 1 for the original theoretical description and refs. 2-4 for experimental details).

The DESY synchrotron uses bursts of 50 pulses/s and each 10 ms pulse contains 6×10^{10} electrons (10 mA average beam current). The injection energy is relatively low and the electrons are accelerated up to 7.5 GeV in the 10 ms.

Most of the X-radiation is emitted during the last 3 ms of each pulse: little radiation is produced at the lower electron energies, and so the time averaged intensity at 1.5 Å is about 20% of the peak value.

Table 1 Data for Quartz Monochromator in Synchrotron Radiation Beam

Synchrotron	7.5 GeV, 10 mA beam current
Electron beam diameter	approximately 4 mm (= effective X-ray source diameter)
Distance	37 m from synchrotron to monochromator
Cross-fire of the incident beam	approximately 10^{-4} rad
Polarization	85% at 1.5 Å in the eighth ms of the cycle, polarized in the plane of the synchrotron
Be-window	0.5 mm (96 mg cm ⁻²)
Crystal	quartz cut at $\sigma = 8^\circ 30'$ to the 1011 planes, dimensions $45 \times 13 \times 0.3$ mm ³
Bender	pins: outer pair 40.5 mm inner pair 39.5 mm radius of curvature of crystal, 9 m
Wavelength	1.53 Å ($\theta = 13^\circ 15'$)
Wavelength spread	$\Delta\lambda = 3 \times 10^{-3}$ Å (due to deviation from Johann focusing and to finite source size)
Focus	1.5 m from crystal line focus 180 µm wide
Angular aperture of reflected beam	horizontal: 2 mrad (convergence) vertical: 3-4 mrad (divergence)
Measured flux in line focus	1.8×10^9 photons s ⁻¹ mm ⁻¹ (of focal length) (at the eighth ms of the cycle)

Table 2 Biological Applications

Specimen	Elliott fine-focus X-ray tube *	DESY synchrotron with Berreman point-focusing monochromator ‡
Single crystal	Standard collimator 0.5 mm diameter	
$a = 0.5$ mm	$A = 12.5$ cm	$D = 1$ m
$b = 0.5$ mm	$d = 0.7$ mm	$d = 120$ µm
$L = 7.5$ cm	$P = 10^9$ photons s ⁻¹	$P = 4 \times 10^9$ photons s ⁻¹
	$I = 2 \times 10^9$ photons s ⁻¹ mm ⁻²	$I = 2.5 \times 10^{11}$ photons s ⁻¹ mm ⁻²
Tobacco mosaic virus gel	Double-crystal focusing monochromator †	
$a = 0.6$ mm	$d = 80$ µm	$D = 0.8$ m
$b = 1$ mm	$P = 10^7$ photons s ⁻¹	$d = 100$ µm
$L = 12$ cm	$I = 2 \times 10^9$ photons s ⁻¹ mm ⁻²	$P = 3 \times 10^9$ photons s ⁻¹
		$I = 3 \times 10^{11}$ photons s ⁻¹ mm ⁻²
Insect muscle	Double-crystal focusing monochromator †	
$a = 3$ mm	$d = 100$ µm	$D = 1.5$ (3) m
$b = 0.3$ mm	$P = 5 \times 10^5$ photons s ⁻¹	$d = 180$ (350) µm
		$P = 5 \times 10^8$ (2 × 10 ⁹) photons s ⁻¹
$L = 40$ cm	$I = 5 \times 10^7$ photons s ⁻¹ mm ⁻²	$I = 1.5 \times 10^{10}$ photons s ⁻¹ mm ⁻²

a , Width of specimen; b , height of specimen; L , specimen film distance; A , anode specimen distance; D , focal length, that is, monochromator film distance; d , spot or focus diameter on film; P , X-ray power reaching the specimen; and I , flux density at the focus.

* Loaded with 40 kV, 50 mA into a 0.2×2 mm² electron focus at the anode in the first case, and 40 kV, 15 mA into a 0.14×0.7 mm² focus in the other two cases. This set is the most powerful fine-focus X-ray tube currently available.

† The setting of this Johann-type⁵ monochromator is optimized for each type of specimen.

‡ Conditions of the synchrotron are as in Table 1, computed for 1.5 Å radiation.

We have evaluated the spectral luminance (that is, the power in photons per second radiated per unit area, solid angle, and wavelength interval) of both the synchrotron and a fine-focus rotating anode X-ray tube (see Table 2). The values are 2×10^{22} (time averaged) and 3×10^{20} photons s⁻¹ sterad⁻¹ cm⁻² Å⁻¹ respectively at 1.54 Å, showing clearly that the synchrotron is, relative to present X-ray tubes, a very bright source. The actual advantage to be gained in a diffraction experiment depends critically on the optical system necessary to focus and monochromate the radiation. Three types of focusing mono-

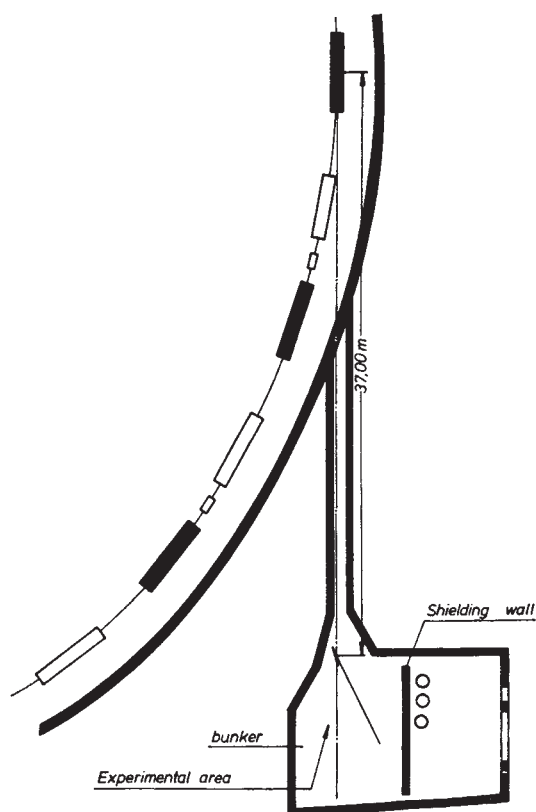


Fig. 1 The F41 bunker at DESY and its position with respect to the synchrotron.

chromators used in normal X-ray diffraction can be used: bent glass mirrors, quartz monochromators and graphite monochromators.

A preliminary investigation of the properties of bent quartz monochromators⁵ used with synchrotron radiation is reported here. We have chosen quartz because of its suitable elastic and optical properties which allow it to be used asymmetrically cut and bent to form an accurate focusing monochromator, with a comparatively large numerical aperture. It also behaves substantially as a perfect crystal with a reflectivity near unity in a narrow angular range. We predict that it should be possible to focus the synchrotron radiation down to a point ($200 \times 200 \mu\text{m}^2$) with a Berreman⁶ monochromator to give a total flux of 10^{10} photons s^{-1} at $1.5 \text{ \AA}$, which is higher than the flux available from other known X-ray sources (Table 2); also the beam is well collimated. The flux density, the important parameter when using film, is comparatively even higher because of the small focus.

Because of its large mosaic spread (300 times greater than that of quartz) a graphite monochromator might seem advantageous for our application. When used with the white radiation from the synchrotron, however, the mosaic spread of graphite would produce a highly divergent reflected beam with considerable wavelength inhomogeneity, thus restricting us to small monochromator-film distances for reasonable spot diameters on the film. For these short distances it would not then be possible to collect radiation from a large area of graphite by focusing. Alternatively, for larger film distances the reflected beam would require collimation, which would again reduce the expected intensity. We do not, therefore, expect graphite to give more intensity than quartz. Furthermore, the optical and mechanical properties of graphite are much less convenient.

Experimental Details

All experiments took place in the F41 (synchrotron radiation) group bunker at DESY in Hamburg (Figs. 1 and 2). The

experimental area can only be entered when the main beam shutter between the synchrotron and the bunker is closed so that all the experiments had to be done by remote control. The quartz crystal was mounted in the vacuum pipe leading to the synchrotron ring. The reflected beam came out through a beryllium window (0.5 mm thick) of diameter 1.5 cm. A rotating disk containing a slot was used as an attenuator. This and a lead shutter were mounted near the window. The rotating disk was arranged to run synchronously with the synchrotron. A film holder was mounted about 120 cm from the quartz crystal on a table movable by remote control. Intensities were recorded on Ilford Industrial G film. The monochromator (Steeg and Reuter) consisted of a slab of quartz ($45 \times 13 \times 0.3 \text{ mm}^3$) with the face containing the long axis cut at about 8° to the 1011 planes. The slab was bent by two sets of pins. Before mounting the crystal in the beam, the curvature was pre-adjusted to the required radius with laser light. The final position of the focus was determined by through-focal photographs. The best focal line had a width of $180 \mu\text{m}$ and represented the image of the radiating electron beam in the synchrotron. (The total effective source size, including the betatron and synchrotron oscillations, was about 4 mm.) Photographs were also taken close to the monochromator, where the reflected beam was wide, to evaluate the total reflected flux. Experiments with aluminium filters were made to estimate the strength of the higher harmonics in the quartz reflected radiation.

With a source-to-monochromator distance of about 40 m the crystal, if set exactly for one wavelength (for example, in the Johann arrangement⁵), would give a focus at 10 m. The white radiation fortunately allowed us to relax this condition and obtain a more practical focal length (1.5 m) at the expense only of very little wavelength inhomogeneity (Table 1). Furthermore, the angular adjustment of the quartz monochromator was not critical. The central wavelength of the reflected beam was determined by measuring the angle between incident and reflected beam. The position and size of the Be-window limited our observations to Bragg angles of $13 \pm 1^\circ$ (that is, $1.5 \pm 0.15 \text{ \AA}$).

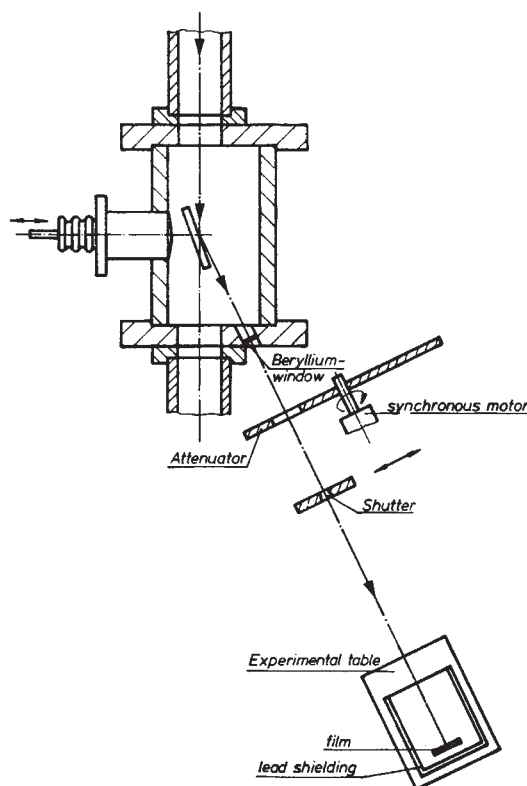


Fig. 2 Monochromator housing and the experimental set-up.

Finally, a simple camera was constructed (specimen-film distance 40 cm), and a photograph (Fig. 3a) was taken of the equatorial reflexions from a 2 mm strip of the longitudinal flight muscle from the giant water bug *Lethoceros maximus*⁷. The entrance aperture of the camera was approximately 2 mm $\times$ 2 mm. A helium-filled tube minimized air absorption in the space between the radiation-pipe window and the camera. The exposure was 15 min with the synchrotron running at 5 GeV. On one side of the direct beam a large area of parasitic scattering is visible apparently resulting from the quartz and from the steel pins used to bend the quartz. Fortunately, the camera entrance-slits were not symmetrically positioned, so that a clear view of one side of the diffraction pattern was obtained. The substantially greater width of the "20" line on the photograph made with synchrotron radiation, compared with that made using a conventional X-ray source (Fig. 3b, Elliott fine-focus rotating anode tube and bent quartz monochromator) has not been explained. The comparative intensity of the two photos shows that the synchrotron (at 5 GeV) is about ten times more effective than one of the most intense X-ray sources currently available.

Calculated and Observed Intensities

Using the theory of Schwinger¹ and a programme written by Klucker, DESY group F41, we have calculated the intensities at 1.5 Å wavelength and at the harmonics of 1.5 Å: when the synchrotron runs at 7.5 GeV the second and third harmonics are twice as intense (photons/s) as the 1.5 Å radiation.

We have measured photographically the instantaneous intensity of the reflected beam passing the disk attenuator at the eighth ms of each synchrotron acceleration cycle. The contribution of higher orders has been estimated from measurements made through aluminium filters of various known thicknesses, and we have adopted values for the absorption coefficients⁸. The sensitivity of Ilford Industrial G film at 1.5 Å has been extrapolated from the calibrated value at 1.54 Å (ref. 9). The experimental conditions and data are summarized in Table 1.

The ratio of the intensity at 1.5 Å, evaluated as indicated above, to the calculated incident intensity per unit wavelength interval is an "integrated band pass" which was found to be

$$\int R(\lambda)d\lambda = 0.7 \times 10^{-4} \text{ Å}$$

Transforming the wavelength into an angle using Bragg's law we find an integrated reflectivity

$$\int R(\theta)d\theta = R_{\text{int}} = 1.0 \times 10^{-5} \text{ rad}$$

for a quartz crystal cut at 8° 30' to the 10 $\bar{1}$ 1 plane.

Quartz behaves essentially as a perfect dynamical diffractor¹⁰. Renninger¹¹ has calculated the reflectivity of a perfect quartz crystal (without corrections for absorption) to be

$$R_{\text{int}} = 4.4 \times 10^{-5} \text{ rad}$$

and Brogren¹² measured an integrated reflectivity of

$$R_{\text{int}} = 3.9 \times 10^{-5} \text{ rad}$$

for a polished quartz crystal cut parallel to the 10 $\bar{1}$ 1 planes. The case of an asymmetrically cut perfect crystal with absorption is treated in the Darwin-Prins theory. Using Zachariasen's formulae¹³ we have calculated an integrated reflectivity of

$$R_{\text{int}} = 1.45 \times 10^{-5} \text{ rad}$$

for a quartz crystal cut at 8° 30' to the 10 $\bar{1}$ 1 planes ($\lambda = 1.5 \text{ Å}$) which agrees with our experimental value.

We emphasize that the aim of our experiments was not to make quantitative measurements of the reflectivity of quartz but to show that quartz is a suitable material for the construction of a focusing monochromator for synchrotron radiation, and to check that there was no large disparity between the observed and calculated flux of monochromated synchrotron radiation. Our results show that the monochromator has

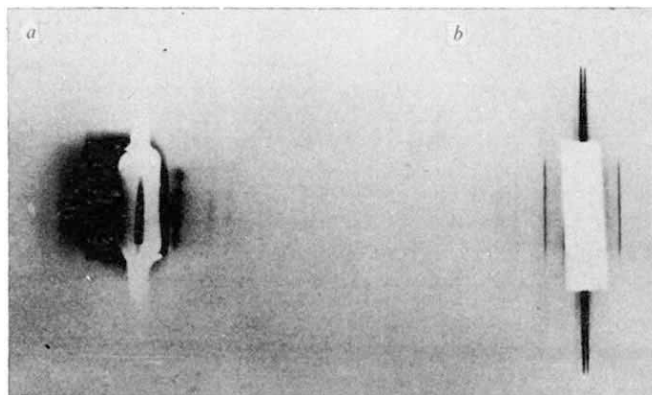


Fig. 3 Equatorial reflexions from dorsolongitudinal flight muscle of *Lethoceros maximus* recorded with: a, monochromated synchrotron radiation; electron energy 5 GeV, beam current 8 mA, exposure time 15 min, specimen film distance 40 cm; note the parasitic scattering on the left of the backstop arising from fluorescence from the monochromator holder; b, Elliott fine-focus rotating anode tube at 40 kV, 15 mA, exposure time 1 h, specimen film distance 36 cm. The strong line is the 20 reflexion ($d = 231 \text{ Å}$); the weak lines are the 21, 31 and 32 reflexions.

properties which can be accurately predicted. We have emphasized neither the accurate determination of the attenuation ratio of the rotating disk nor the speed of the shutter. Moreover, the evaluation of the contribution from higher harmonics may be inaccurate. We estimate that the error in our result may amount to 50%. Furthermore, the state of the surface of the quartz crystal is difficult to control, although it has a considerable influence on the actual shape and height of the reflectivity curve^{14,15}.

Estimated Intensities for Various Configurations

We intend to set up a Berreman monochromator⁶ to give a point-focused beam from a quartz crystal ground so as to give the required curvature in one plane and bent to the corresponding curvature in the second. There seem to be no theoretical reasons why this should not produce foci of similar dimensions to those that we have obtained with a simple bent crystal, especially as the geometry of the synchrotron beam relaxes some of the stringent conditions which the radii of curvature of the crystal must otherwise satisfy.

The estimated performance of such an arrangement for each of three typical configurations used in biological applications of X-ray diffraction is shown in Table 2, and the performance is compared with a "conventional" fine-focus rotating anode tube. The calculated intensities are based on the effective band pass given above, $0.7 \times 10^{-4} \text{ Å}$.

The tube values were calculated from measurements made with Ilford Industrial G film and a rotating disk attenuator on an Elliott fine-focus rotating anode tube used with single and double focusing quartz monochromators.

Higher Intensities and Longer Wavelengths

Some possible methods of obtaining higher intensities and utilizing the continuous spectrum are as follows. (a) According to current plans, the DESY synchrotron current will be raised from 10 mA to 50 mA. Also the electrons will be kept at the maximum energy for 1 or 2 ms, giving overall a six-fold improvement. (b) Sakisaka¹⁴ suggests that both the height and width of the rocking curve of quartz can be increased appreciably by gentle grinding. A gain of 2 or 3 should be possible without affecting the size of the focus. (c) For special applications, where only pulses of X-rays can be used, the synchrotron is a very advantageous source if the experiment can be synchronized with the periodic maximum emission from the

synchrotron. The integrated reflectivity of quartz increases approximately linearly with wavelength up to 3–4 Å (ref. 12). The intensity of the synchrotron radiation decreases, however, in the wavelength range 1.5–4.5 Å, approximately as the inverse of wavelength. The reflected intensity is thus roughly independent of wavelength. Previously, long wavelength experiments were avoided because of the low conversion efficiency of the anode materials involved.

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Molecular Packing in Collagen

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X-ray diffraction suggests an intermediate level of organization in collagen fibrils: a grouping of the tropocollagen molecules into five-stranded ropes.

THE geometry of the three dimensional molecular packing in collagen fibrils is still unknown. It is usually accepted that collagen is composed of tropocollagen molecules 2900 Å long, 15 Å wide and with a triple-helical conformation. Information about the packing of these molecules has come from electron microscopy and the meridional X-ray pattern, and has led to two dimensional models, such as the quarter-stagger sheet¹. The equatorial X-ray pattern, which would give fuller information about the lateral packing, is unclear, although there is a study by North *et al.*². We re-describe here this pattern and discuss some immediate implications.

Fig. 1 shows typical medium angle X-ray patterns of rat tail tendon taken in conditions designed to preserve the native state. The principal features, and our interpretation of them, are as follows.

(a) As North *et al.* observed, the region of high intensity on the equator at 13 Å, usually associated with interference between neighbouring tropocollagen molecules, is broken up into a triplet by splits in directions both parallel to and perpendicular to the fibre axis.

(b) Between this triplet and the origin are further sharp reflexions, associated with row lines which sample the region out to, and including, the 10 Å layer line. This sampling of characteristic collagen helix features at relatively low values of *R*, the distance from the meridian, suggests a grouping of tropocollagen molecules.

(c) The *R*-sharpness of these reflexions indicates that these groups lie on a lattice which extends laterally for at least 1000 Å in each fibril. Their sharpness relative to their separation, and the admixture of equatorial and off-equatorial reflexions,

mak : explanations^{3,4} in terms of limited size aggregates unconvincing. It seems more likely that, for most of the spots, the entire fibril (in this case mostly 2000–3000 Å in diameter) diffracts coherently.

(d) Some of the reflexions reported by North *et al.* are not observed in our patterns; or, like the 49 Å equatorial, are apparently due to lipid. Thus their indexing on the basis of a monoclinic cell is implausible.

(e) Three of the reflexions can be indexed as the orders of 38 Å, so that the prominent lateral repeat distance in the fibril is 38 Å.

(f) After the fibres were treated with dilute solutions of heavy metal stains, the intensity of the 38 Å row line increased. Stain, therefore, seemed to have outlined an object of this lateral dimension, suggesting that the repeat is not merely geometrical but reflects a physical grouping.

(g) The intensity on the 38 Å row line consists of a large number of sharp spots which are not, however, on the layer lines of roughly 640 Å spacing, defined by the meridional series. This fine structure on a non-meridional row line shows that the fibril has at least the elements of full three dimensional order, not merely the statistical regularity in the axial direction proposed by Grant *et al.*⁵.

(h) Because there are no reflexions, even off the equator, to indicate a lateral periodicity of more than 38 Å, the layers must be individually 640 Å-periodic in axial projection (that is, the overall 640 Å period cannot be generated by the axial staggering of non-640 Å-periodic layers).

(i) There is a complex group of equatorial and near-equatorial reflexions at about 24 Å which neither fall on the 38 Å series nor are related to it in any simple way, but which again are associated with a row line which samples the collagen transform. This suggests a grouping in other directions also, forming bundles.

(j) The profile of intensity in the equatorial region, considered either for the diffuse background or the transform "underlying" the sharp reflexions, suggests that the object on the lattice is a small bundle of tropocollagen molecules.

(k) Both the 10 Å layer line and the equator show a pronounced fanning of the intensity distribution. Some of the sharp reflexions in both regions are on this fan, and the very strong reflexion at 13.5 Å is sharply split across the equator. The splitting corresponds to an axial spacing of about 200 Å. Because of the fanning, this strong intensity away from the ideal equator and 10 Å layer line must result from tilting of the tropocollagen units, rather than from a short coherent length of the contributing molecular segments.

(l) The sharpness in the Z (fibre axis) direction of the 2.9 Å meridional is comparable with that of the 13.5 Å off-equatorial, suggesting that a tilted or supercoiled geometry of a rather uniform triple-helical secondary structure is maintained over at least 200 Å.

(m) As well as the reflexions associated with row lines, there are peaks, such as those at 17.3 Å and the strong equatorial component at 12.6 Å, which lack definite indications of a row line. But the intensity of the reflexions of all types is reduced greatly in the region around 7–8 Å on the equator, where the transform of the collagen triple-helix is characteristically very weak: thus it is likely that even those reflexions not associated with row lines affect the collagen packing arrangement.

(n) So far we have, for simplicity, ignored a further complication: the three row lines at 38 Å, 24 Å and 19 Å are split, each comprising two row lines crossing at a small angle, roughly 3°, at the equator. This suggests *a priori* that the meridian, or zero-order row line, should exhibit a similar splitting, implying that no intensity should appear anywhere exactly on the meridian. In most patterns the higher order 640 Å meridional spots do get broader perpendicular to the meridian, the splitting in the centre being hard to observe. The form of this splitting is similar to that of the diffraction by a sheared diffractor⁶ (although our observations relate to the native undried material). In the real fibril, the effect suggests an axial shearing of a three dimensional crystal, or a relative axial displacement of successive layers in a cylindrical lattice, or an axial screw dislocation. It is not characteristic of a distortion in which the motion of scattering matter is entirely perpendicular to the fibre axis.

(o) Our studies of fibres treated in different ways show that the native (crossed row line) structure can be experimentally modified to yield an analogous arrangement, in which all meridional reflexions are closely confined, and the row lines, although still sharp and at the same *R*-values, are now exactly vertical. In this arrangement, which we name orthomorphic, the general intensity profile, although not the exact distribution of sampling positions, is unchanged in the equatorial and 10 Å layer line regions, indicating that the tilting or supercoiling of the molecules is not structurally linked to the tilt of the row lines.

(p) This tilting of the row lines implies that in some sense the banding of the fibril in the native state is not exactly transverse, but is inclined to the transverse plane at about 3°. Electron microscopy has not in general indicated this. The discrepancy might be removed by our observations that when the fibres were subjected to an analogue of the electron microscopy staining procedure using higher concentrations than in (f), a transition occurred similar to, but more extreme than, the orthomorphic; the meridional reflexions were sharply confined to the axis, corresponding to a true transverse banding.

We now have to propose a model for the packing arrangement which incorporates these conclusions. Our X-ray results suggest that this can be done geometrically in terms of groups of molecules arranged on a quasi-regular lattice; but before identifying these groups as, for example, microfibrils, we would like to know their physical significance. Attempts at formal model building made so far are suggestive here. The 640 Å period is usually "explained" by a modified quarter-staggered sheet¹, but our results disagree with any type of stacked-sheet arrangement. Veis *et al.*⁷ proposed a microfibril structure which could explain the 640 Å period if a

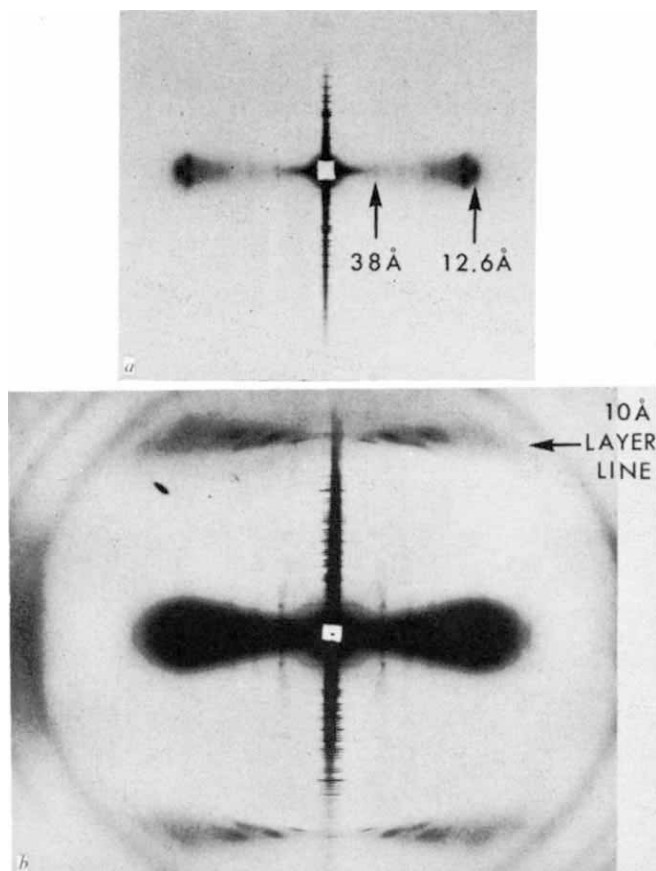


Fig. 1 Medium angle X-ray diffraction patterns of rat tail tendon, with exposure times of (a) 12 h, (b) 90 h, fibre axis vertical, specimen-to-film distance 17 cm. Pictures were taken using an Elliott rotating anode source, and a combined mirror/monochromator focusing camera. The specimen, after brief immersion in 0.5 M NaCl, was held under slight tension over water in a closed cell. The salt treatment had no effect on the diffraction pattern, and allowed long exposures to be taken with the water content of the specimen remaining constant.

specific staggering of microfibrils were also assumed although this and the small diameter (32 Å) are again inconsistent with our results, as is, similarly, a proposed two-strand rope⁸. Smith⁹, however, modified the sheet structure by wrapping it round to form a five-stranded rope, and we consider that, as far as it goes, this may explain all the X-ray features. Apart from these and other X-ray arguments, the overwhelming attraction of Smith's model is the simple interpretation that it makes of the 640 Å periodicity, irrespective of the way in which the ropes are, in turn, packed together. Ropes with a larger number of strands could also have a 640 Å repeat, but they would not have the unstained "bands" observed in negatively stained fibrils. The five-stranded rope thus acquires the status of a microfibril because of its physical integrity. That such a microfibril exists is evident from the electron microscope data which indicate the presence of lateral groupings distinctly larger than single molecules¹⁰, and from the fact, illustrated by our orthomorphic transition, that the 38 Å units can move past each other axially while retaining their internal packing arrangement of tropocollagen units. We note that the five-stranded rope, but not the sheet structure or Veis's four-stranded rope, has the feature that the molecules are related as subunits on a helix; this implies that the axial periodicity is generated by a one-stage aggregation, which has advantages already recognized in a self-assembling system¹¹.

Even if we accept Smith's basic idea, several points remain unsettled, such as whether the molecules are straight or coiled, the pitch of the genetic helix and the absolute hand of the stagger helix and of the supercoil, if present. We have also to determine the packing geometry of the microfibrils. Here we

will consider only the principal question of whether the microfibrils are straight, but inclined at a constant angle to the axis, or supercoiled, with a rather constant pitch. Only one of these situations can account for the sharp splitting across the equator at 13.5 Å. These are not easily distinguishable on X-ray evidence, but we believe that the straight-but-tilted model makes specific predictions for the sampling positions in the 10 Å layer line and equatorial regions, which are not fulfilled. A modified form of the straight-tilted arrangement was proposed by Chapman¹²; in this structure the molecules are inclined in tangential but not in radial planes, to an extent that varies with the distance from the fibril axis. We would modify the proposal of Chapman by saying that the tilted unit is our five-stranded rope rather than the single molecule, but even then we feel that the requisite continuous range of molecular tilts is not observed, and conclude that the explanation of the entire pattern must be in terms of a laterally packed arrangement of five-stranded "coiled coiled coils", each of which runs through the fibril parallel to the axis, with only a small departure from the alignment of their respective gap/overlap patterns in axial (though not necessarily azimuthal) register.

We cannot define the lattice of microfibrils because we have not yet indexed the reflexions, but if we are correct in concluding that the microfibril is a five-stranded rope we can refer to discussions of rope packing by, for example, Caspar *et al.*¹³ (pages 101–2) and Elliott and Lowy¹⁴ (page 192). Caspar *et al.* cite, as a possible cause of the unusual packing in tactoids, the discrepancy between stagger and coiled-coil periodicities. But this discrepancy is not inevitable, because the optimum rope interlocking can always be achieved, whatever the axial stagger, by appropriate rotation about the rope axis. Thus in paramyosin the optimum relationship (body-centring) could result if neighbours were related by a 90° azimuthal rotation, without axial displacement. We would expect, similarly, a relative rotation of neighbours for collagen, but we cannot define it further when we consider the five-strandedness and such

additional features as the gap regions and the surface distribution of "knobs and holes".

Diffraction by such a system has some interesting features. It can be shown that the Bragg reflexions are flanked by sharp satellites, whose positions depend on the magnitude of the relative rotation. For such a complex object as the microfibril, the parts of the structure of different symmetry, such as the five-fold rope of tropocollagen molecules and the helical distribution of gaps, have to be considered separately.

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Structure and Activity of Muscarinic Stimulants

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Crystalline, solution and theoretical conformational analyses of stimulants of the autonomic post-ganglionic parasympathetic (muscarinic) nervous junction show a strong correlation between three-dimensional structure and activity.

INTERCELLULAR communication in the nervous system is effected by the release from a nerve cell of a transmitter

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substance which diffuses across a synaptic space and stimulates the next cell, either another nerve cell or an organ. The natural transmitter substance of the post-ganglionic parasympathetic junction between the parasympathetic nerves of the autonomic nervous system and the autonomic organs is acetylcholine¹, $(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CH}_2\text{O}(\text{CO})\text{CH}_3$. This junction is known as the muscarinic junction, because the natural substance muscarine from the mushroom *Amanita muscaria* simulates the effect of acetylcholine². Such mimics which cause an observable effect are termed agonists of the system. For more than 20 yr³, attempts have been made to determine the structure-activity relationships of cholinergic agonists without experimental conformational information. X-ray diffraction, supplemented by conformational analyses in solution and by calculation, has now made it possible to

make a reasonable correlation of conformation with activity for a large class of muscarinic agonists and to determine the probable groups associated with interaction with the receptor.

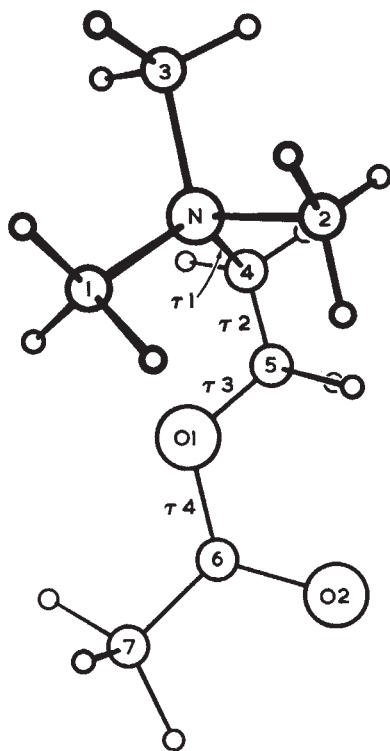


Fig. 1 A perspective drawing of the molecule of acetylcholine, showing atomic numbering and the four torsion angles which are the four parameters of the conformation of the molecule. The conformation shown is that proposed as relevant to the muscarinic receptor and is similar to that observed in crystals of the chloride.

Acetylcholine

The acetylcholine molecule (ACh) is labile with several stable conformations⁴⁻⁷. Accepting known values for interatomic bond distances and bond angles, and neglecting the orientations of the methyl groups, the conformation of ACh is completely described by the four torsion angles (ref. 8) shown in Fig. 1. (The torsion angle τ of the bonded group X-A-B-Y is the angle between the plane X-A-B and the plane A-B-Y. It is positive ($0 \rightarrow +180^\circ$) if clockwise and negative ($0 \rightarrow -180^\circ$) if anticlockwise from the near atom X to the further Y. Values of $\pm\tau$ of 0° , 60° , 120° and 180° are termed $\pm$, synplanar, synclinal, anticlinal and antiplanar, respectively.) The conformation with three methyl groups in the staggered conformation (τ_1 referred to C3 = 180°) is more stable than the eclipsed ($\tau_1 = 0$ or 120°) by approximately 12 kJ mol^{-1} (ref. 9) and because only the staggered conformation has been observed in the known crystal structures of related molecules, we accept τ_1 to be approximately 180° . The trigonal nature of the carbonyl carbon atom C6 and the partial double bond character¹⁰ of the C6-O1 bond, makes the acetoxy group planar with $\tau \text{ O2-C6-O1-C5} = 0^\circ$ and $\tau \text{ C7-C6-O1-C5} = 180^\circ$. The resonance energy¹⁰ stabilizing the planar conformation of esters is 100 kJ mol^{-1} , too high to be affected by any interaction between the molecule and a receptor. The two torsion angles, τ_1 and τ_4 , almost certainly persist in solution or after interaction with a receptor substance. Values of the two remaining torsion angles, τ_2 and τ_3 , for that conformation of acetylcholine relevant to interaction with the muscarinic receptor can be determined by reference to the observed conformations of other more rigid muscarinic agonists.

A van der Waals energy conformational analysis of ACh shows that the molecule has seven possible conformations of nearly equal energy⁷. A molecular orbital energy analysis¹¹ indicated the τ_2 synclinal τ_3 antiplanar conformation is most stable. Crystal structure analyses of ACh and its derivatives show five of these conformations. In the chloride⁵ and the tri-iodo mercuric compounds (R. W. B. and N. Datta, unpublished work) ACh is synclinal-antiplanar (Fig. 2) and, in the bromide, synclinal-synclinal⁴. In solution, the observed conformation is synclinal-antiplanar¹², which is presumably that of lowest energy in hydrophilic media. The lowest energy conformation suggested by a van der Waals calculation⁷ is antiplanar-antiplanar, but this calculation ignores the $\text{N}^+-\text{O}\delta^-$ electrostatic interaction. Of thirty-two known crystal structures of $(\text{CH}_3)_3\text{N}^+-\text{C}-\text{C}-\text{O}$ groups, twenty-eight are synclinal at τ_2 and four are approximately antiplanar, the latter because of hydrogen-bonding or crystal packing forces.

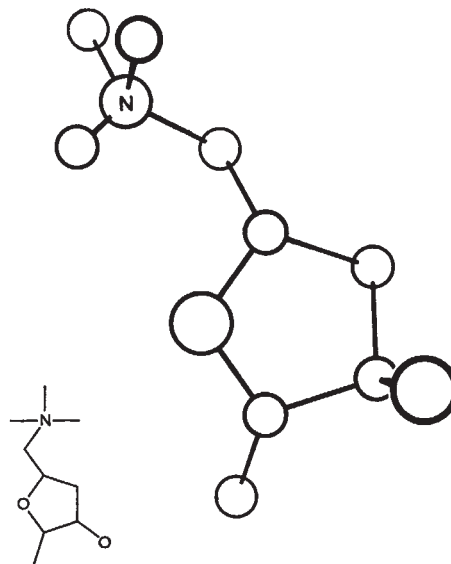


Fig. 2 L(+)-Muscarine as in crystals of the iodide¹³ projected onto the plane of the coplanar atoms of the tetrahydrofuran ring.

Conformational Correlation of Potent Agonists

We shall review here first the conformations observed in crystals, in solution and derived by theoretical analyses for all potent muscarinic agonists of which the crystal structures are known. We shall then compare the observed conformations, and determine the conformation or range of conformations, the pharmacodynamic groups and absolute configuration which are required for potent muscarinic activity, and discuss small variations of conformation from those observed which give greater consistency of conformation. Finally, we shall discuss some weakly active or inactive compounds.

L(+)-Muscarine and Analogues

L(+)-Muscarine iodide¹³ has the conformation shown in Fig. 2 in crystals of the iodide with the conformational parameters given in Table 1. The equipotent molar ratio (EPMR) is shown in terms of the number of molecules required to equal the effect of acetylcholine, without correction for the rate of hydrolysis by acetylcholinesterase. The conformation is synclinal at $\tau_2 = +73^\circ$ and $\tau_3 = +144^\circ$. The ring is in the envelope conformation, with one atom C9 in the plane of the antiplanar extended chain C3-N-C4-C5. This conformation of muscarine is the most stable on the basis of van der Waals energy calculations¹⁴ and molecular orbital calculations¹¹.

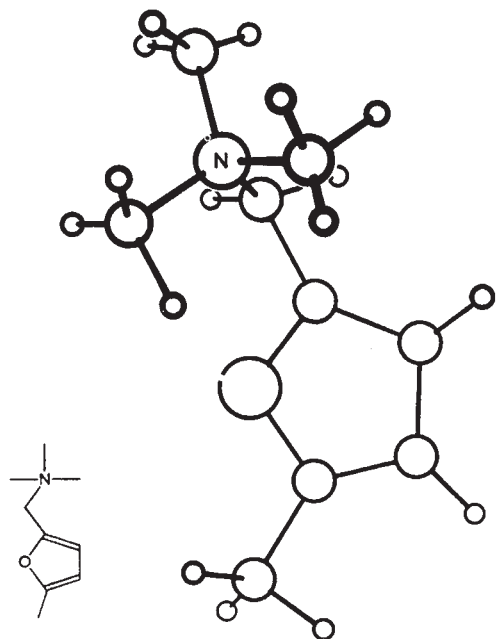


Fig. 3 5-Methylfurfurmethide as in crystals of the iodide projected onto the plane of the furan ring.

The observed conformation of the most potent isomer and enantiomer of F2268, *L*(+)-*cis*-2*S*-methyl-4*R*-trimethylammoniummethyl-1,3-dioxolan¹⁵, in crystals of the iodide¹⁶ is very similar to that of muscarine (Table 1). The only difference is that the out of plane ring atom is on the opposite side of the plane of the ring in the 1,3-dioxolan, resulting in $\tau_3 = +103^\circ$.

The fairly rigid potent muscarinic molecule 5-methylfurfurmethide¹⁷ has the conformation shown in Fig. 3 in crystals of the iodide (R. W. B., C. H. C. and P. J. P., results to be published). τ_3 and τ_4 are fixed at 180° by the double-bonds of the planar ring system, and the conformation has only one parameter, τ_2 , which is observed at $+81^\circ$ and $+83^\circ$ in the two molecules in the asymmetric unit and is restricted to near this value by repulsions between the trimethylammonium group and the ring.

Derivatives of Acetylcholine

The conformation of the potent muscarinic agonist *L*(+)-*S*-acetyl- β -methylcholine (EPMR = 1)¹⁸ in crystals of the iodide¹⁹ (Fig. 4 and Table 1) is synclinal at τ_2 and $\tau_3 = -147^\circ$, because of close contact between the β -methyl group (C8) and the carbonyl oxygen atom (O2). This conformation is the same as that observed in solution²⁰. One of the two conformations of *D*(+)-*R*-acetyl- α -methylcholine (EPMR = 28)¹⁸ observed in crystals of the iodide²¹ is similar to that of acetyl- β -methylcholine iodide and to ACh in crystals of the chloride. It is synclinal at τ_2 and antiplanar at τ_3 (Table 1). The other conformation of acetyl- α -methylcholine observed in these crystals with $\tau_2 = -148^\circ$ is similar to another conformation calculated to be stable⁷ and is the mirror image of the conformation of substrates considered to be relevant to interaction with the hydrolysing enzyme, acetylcholinesterase²². The structure²³ of the potent muscarinic agonist *erythro*-

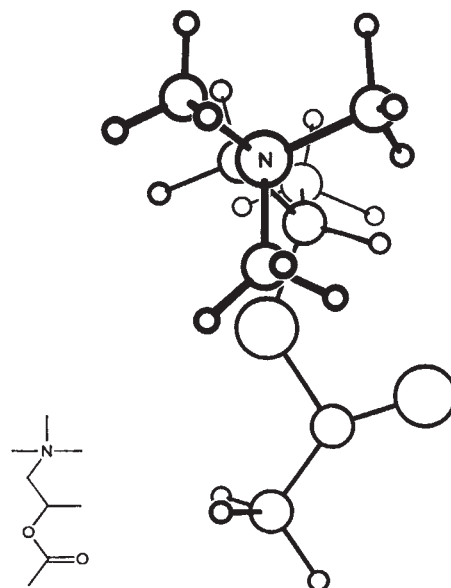


Fig. 4 *L*(+)-*S*-Acetyl- β -methylcholine as in crystals of the iodide¹⁹ projected onto the plane of the acetoxy group.

Table 1 Certain Torsion Angles and Interatomic Distances observed in Crystals of Potent Muscarinic Agonists

Compound	τ_1	τ_2	τ_3	τ_4	N ⁺ -O1	N ⁺ -O2	N ⁺ -C6	N ⁺ -C7	EPMR
Acetylcholine bromide	-176	+77	+79	-167	329	442	412	507	1
Acetylcholine chloride	+171	+85	-167	-175	326	480	440	538	1
<i>L</i> (+)-muscarine iodide	-175	+73	+144	-136	307	565	449	540	0.33
<i>L</i> (+)- <i>cis</i> 2(<i>S</i>)-Methyl-4(<i>R</i>)-trimethylammoniummethyl-1,3-dioxolan iodide	+150	+94	+103	-132	320	479	446	484	0.17
5-Methylfurfurmethide iodide <i>a</i>	+167	+83	+174	+179	326	—	456	564	1
<i>b</i>	+169	+81	+176	+178	322	—	451	563	1
<i>L</i> (+)- <i>S</i> -Acetyl- β -methylcholine iodide	-175	+87	-143	177	321	439	425	516	1
<i>D</i> (+)- <i>R</i> -Acetyl- α -methylcholine iodide <i>a</i>	-179	+89	+167	-177	317	496	440	546	28
<i>b</i>	-172	-150	-179	+177	365	518	496	603	28
<i>erythro</i> -Acetyl- α (<i>R</i>), β (<i>S</i>)-dimethylcholine iodide	-179	+76	-155	+173	307	465	410	493	7
Carbamoylcholine	+174	+178	-174	-165	369	508	482	596	1
(+)- <i>trans</i> -2(<i>S</i>)-Acetoxy-cyclopropyl-1(<i>S</i>)-trimethylammonium iodide	+160	+137	+147	-179	369	511	500	611	0.9

$\tau_1 = \text{C5-C4-N-C3}$; $\tau_2 = \text{O1-C5-C4-N}$; $\tau_3 = \text{C6-O1-C5-C4}$; $\tau_4 = \text{C7-C6-O1-C5}$; torsion angles in degrees; distances in pm. References to structures and equipotent molar ratio with respect to acetylcholine are given in the text.

acetyl- α,β -dimethylcholine iodide²⁴ (EPMR = 7, but the compound is not hydrolysed by AChase²⁴) is very similar to the observed conformation of acetyl- β -methylcholine.

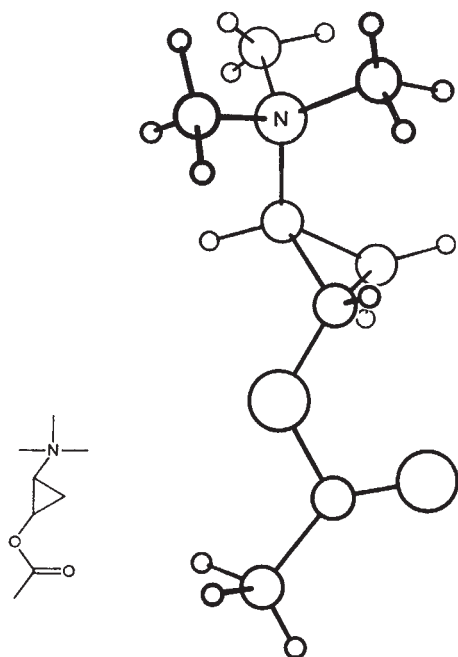


Fig. 5 (+)-*trans*-2S-Acetoxy-cyclopropyl-1S-trimethylammonium as observed in crystals of the iodide²² projected onto the plane of the acetoxy group.

The conformation of the muscarinic agonist carbamoylcholine¹ in crystals of the bromide²⁵ is the only known example of a potent muscarinic agonist antiplanar at $N^+-C-C-O$, $\tau_2 = 178^\circ$. The conformation is stabilized by several hydrogen bonds to the carbamate group. In solution, carbamoylcholine is synclinal at τ_2 (J. Feeney and P. Partington, results to be published). The 5,6-dimethyl-4 phenyl derivative of carbamoylcholine is synclinal at $\tau_2 (+81^\circ)$ and antiplanar at $\tau_3 (+159^\circ)$ in crystals of the bromide as is the 4-methyl derivative ($\tau_2 = 73^\circ$ and $\tau_3 = 172^\circ$)^{26,27}. The phenyl derivative of carbamoylcholine bromide²⁶ is antiplanar at $\tau_2 = +152^\circ$ and anticlinal at $\tau_3 = -106^\circ$. The conformation of carbamoylcholine must be determined by reference to more rigid agonists, as must that of acetylcholine relevant to muscarinic receptors.

Acetoxy-cyclopropyl-trimethylammonium

The conformation and absolute configuration of the more potent enantiomer of *trans*-acetoxy-cyclopropyl-trimethylammonium²⁸ (ACTM) iodide have been determined²⁹ (Table 1). This conformation is an upper limit of observed values of potent muscarinic agonists at τ_2 of $+137^\circ$ which is fixed by the rigidity of the cyclopropane ring. Accepting the antiplanar conformation of τ_1 , the conformation of this molecule has only one variable, that is the value of $\tau_3 = -151^\circ$, which is similar to that observed in acetyl- β -methylcholine.

No single conformation can describe all known potent muscarinic agonists³⁰. For example, τ_2 is fixed at $+137^\circ$ in ACTM and cannot be greater than about $+120^\circ$ in 5-methylfurfmethide because of steric hindrance between the trimethylammonium group and the ring. τ_3 cannot be more antiplanar than about -160° in acetyl- β -methylcholine, because of hindrance between the β -methyl group (C8) and the carbonyl oxygen atom (O2). But τ_3 must be 180° in 5-methylfurfmethide and approximately $+144^\circ$ in muscarine because of the ring structure. τ_4 is necessarily 180° in ACh and 5-methylfur-

methide because of the trigonal planar nature of the double bonded C6, but must be about -137° in muscarine and the 1,3-dioxolan because of the tetrahedral nature of C6 and the ring structure of these compounds. We can make certain generalizations, however. Excepting the conformation of ACh in crystals of the bromide, the 1,3-dioxolan and carbamoylcholine (the conformation of which will be described later), all the observed conformations can be described by: $\tau_1 = 180^\circ$; $\tau_2 = 103 \pm 34^\circ$; $\tau_3 = 180 \pm 36^\circ$; and $\tau_4 =$ either 180° or -137° . A relationship between τ_2 and τ_3 , however, produces a closer relative relationship among the proposed pharmacodynamic groups: as $\tau_2 = +103^\circ \pm \Delta$, then $\tau_3 = 180^\circ \pm \Delta$, remembering that torsion angles less than 180° are positive ($O \rightarrow +180^\circ$) and those greater than 180° are negative ($O \rightarrow -180^\circ$).

Pharmacodynamic Groups

Neither choline nor acetic acid is very active as an agonist, therefore the groups required for muscarinic activity must include parts of both the choline and the acetoxy moieties of the molecule of ACh. The trimethylammonium group is required for potent muscarinic activity³¹, although the substitution of one *N*-ethyl group does not greatly decrease activity¹. The high muscarinic activity of choline ethyl ether ($(CH_3)_3N^+CH_2CH_2OCH_2CH_3$) and of 5-methylfurfmethide¹, which do not contain an oxygen atom corresponding to the carbonyl oxygen atom (O2), indicates that this atom is not necessary for potent muscarinic activity. In substances which do have a second oxygen atom (O2) such as ACh, muscarine, the 1,3-dioxolan and pilocarpine, the distances between N^+ and this oxygen atom vary by 140 pm, much more than is required for a close fit between agonist and receptor (which should vary by a maximum of 50 pm³²). The acetoxy methyl group (C7), or some smaller group such as NH_2 as in the case of carbamoylcholine, is common to all potent muscarinic agonists and seems to be essential for muscarinic activity.

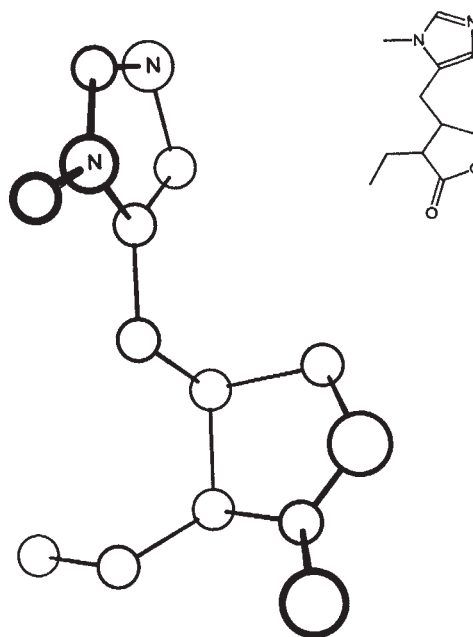


Fig. 6 The weakly active molecule pilocarpine¹ as observed in crystals of the trichlorogermanate⁴⁰.

The ester oxygen atom (O1) is common to all the potent muscarinic agonists considered here, but is not part of some weaker agonists such as pilocarpine. The potent muscarinic agonist *n*-pentyltrimethylammonium (EPMR = 8 on guinea-pig ileum¹) contains no oxygen atoms, and thus neither oxygen

atom is essential for its activity. A potent central nervous system muscarinic agonist, oxotremorine³³, contains no oxygen atom equivalent to O1, the chief role of which may be to hold the N⁺-C-C-O chain in the synclinal conformation. The pharmacodynamic groups required for activity are thus the trimethylammonium group (CH₃)₃N⁺ and the methyl group C7; the ester oxygen atom seems to increase activity and the carbonyl oxygen atom O2 is not necessary.

groups given here better if their conformation on interaction with the receptor were slightly modified from that observed in crystals. The active isomer and enantiomer of F2268, L(+)-*cis*-2*S*-methyl-4*R*-trimethylammoniummethyl-1,3-dioxolan, have $\tau_3 = +103^\circ$ in crystals of the iodide¹⁶ because the out of plane ring atom is on the side opposite to the other substituents. If this atom were on the same side as the other substituents, as is observed in L(+)-muscarine, a conformation of almost equal

Table 2 Certain Torsion Angles and Interatomic Distances observed in Crystals of Some Weakly Active or Inactive Muscarinic Agonists

Compound	τ_1	τ_2	τ_3	τ_4	N ⁺ -O1	N ⁺ -O2	N ⁺ -C6	N ⁺ -C7	EMPR
Pilocarpine trichlorogermanate <i>a</i>	—	—	—	—	511	644	545	571	~100
<i>b</i>	—	—	—	—	505	629	542	565	~100
Arecoline hydrobromide <i>a</i>	—	—	—	—	426	474	374	614	~100
<i>b</i>	—	—	—	—	428	490	384	618	~100
Acetylthiocholine bromide	—	171	129	150	—	—	—	—	320
Acetylselenocholine iodide	—	175	124	155	—	—	—	—	220
(-)-R-3-Acetoxy-quinuclidine methiodide	-173	+107	+76	+179	340	452	433	549	600
2(S)-Trimethylammonium-3(S)-acetoxy- <i>trans</i> -decalin iodide	—	+147	-90	—	—	—	—	—	1,700
<i>threo</i> -Acetyl- α (S), β (S)-dimethylcholine iodide	+178	+143	-95	+175	368	383	412	540	2,800
Lactoylcholine iodide	+176	+85	+157	+173	319	515	456	544	850

Muscarinic agonists are clearly very stereospecific, which could imply at least three pharmacodynamic groups. The stereospecificity may arise, however, from both the essential groups and the supporting groups³⁴. The essential pharmacodynamic groups must be in the correct relative position and the supporting structure must not interfere with the agonist—receptor interaction. The supporting structure of the agonist must contain “holes” (the absence of groups which interfere with the interaction). Thus, the stereospecificity of muscarinic agonists does not necessarily imply the existence of three essential pharmacodynamic groups interacting directly with the receptor. Two essential groups suffice, together with the requirement that no substituents of the supporting group interfere with the agonist—receptor interaction.

Absolute Configuration of Muscarinic Agonists

Table 3 lists torsion angles (τ_2) and equipotent molar ratios compared with acetylcholine of the potent muscarinic agonists for which enantiomers have been separated and tested pharmacologically. Except for muscarone which has approximately equal activities¹ for the two enantiomers and thus provides no stereospecific information, the absolute configuration of the more potent enantiomers of muscarinic agonists is entirely consistent. In all cases, the torsion angle τ_2 of the more potent enantiomer lies in the range $+73^\circ$ to $+137^\circ$.

All the figures show muscarinic agonists in the absolute configuration required for potent muscarinic activity. As originally reported³⁵, the absolute configuration of the more potent enantiomer of 3-acetoxy-quinuclidine methiodide is incorrect but has subsequently been corrected³⁶. This stereospecificity of muscarinic agonists can be compared with the lack of stereospecificity of nicotinic agonists³⁷ caused by the different pharmacodynamic groups involved in interaction with the two types of receptor^{34,38}.

Variations on the Theme

Certain compounds would fit the conformational rules and the relative dispositions of the pharmacodynamic

Table 3 Observed Torsion Angles τ_2 and Equipotent Molar Ratios of Separated Enantiomers of Muscarinic Agonists

Enantiomer	τ_2	EMPR	EMPR ref- erence
L(+)-Muscarine	+73	0.33	1
D(-)-Muscarine	-73	130	1
L(+)-Muscarone		0.15	1
D(-)-Muscarone		0.063	1
L(+)- <i>cis</i> -2(S)-Methyl-4(R)-trimethylammoniummethyl-1,3-dioxolan	+94	0.17	15
D(-)- <i>cis</i> -2(R)-Methyl-4(S)-trimethylammoniummethyl-1,3-dioxolan	-94	17	15
L(+)-S-Acetyl- β -methylcholine	+85	1	18
D(-)-R-Acetyl- β -methylcholine	-85	240	18
D(+)-R-Acetyl- β -methylcholine	+90	28	18
L(-)-S-Acetyl- α -methylcholine	-90	232	18
(+)- <i>trans</i> -2(S)-Acetoxy-cyclopropyl-1(S)-trimethylammonium	+137	0.9	28
(-)- <i>trans</i> -2(R)-Acetoxy-cyclopropyl-1(R)-trimethylammonium	-137	453	28
(-)-R-3-Acetoxy quinuclidine methiodide	+106	600	40
(+)-S-3-Acetoxy quinuclidine methiodide	-106	36,000	40

EMPRs are not corrected for the effects of acetylcholinesterase.

energy³⁹, τ_3 would be about $+145^\circ$ as in muscarine and the conformation would be identical to muscarine. L(+)-S-Acetyl- β -methylcholine and *erythro*-acetyl- α , β -dimethylcholine were observed with τ_3 approximately -150° . The relationship between the proposed pharmacodynamic groups (CH₃)₃N⁺ and CH₃ (C7) would be improved if τ_2 increased to between $+100$ and $+135^\circ$. This increase is possible, as shown by the high activity of ACTM in which τ_2 is fixed at $+137^\circ$. Carbamoylcholine in crystals of the bromide is the only muscarinic

agonist observed to have an antiplanar conformation²⁵. In solution, it is synclinal at τ_2 and so is similar to other muscarinic agonists.

Weakly Acting and Inactive Substances

The structure of the weakly acting (EPMR=100–300) partial agonist (maximum response=0.7 that of ACh) pilocarpine¹ has been analysed in crystal of the trichlorogermanate⁴⁰. The only features that this compound and the other agonists considered have in common are the nitrogen atom, the methyl group (C1) and the methyl group (C7). The distance between the nitrogen atom and the methyl group (C7) is similar to that in other agonists (Table 2). The activity of pilocarpine may be mostly attributable to ganglion stimulation⁴¹. The structure of the weakly active muscarinic agonist arecoline¹ (P. J. P. and T. J. P., work to be published) is sufficiently unlike the other agonists considered to account for its low activity. The relative orientation of the $(\text{CH}_3)_3\text{N}^+$ group and $\text{CH}_3(\text{C7})$ differs from the other agonists considered, but the distance between them is similar. The activity of arecoline may be indirect^{1,41}. The muscarinically, weakly active²⁴ substrates of acetylcholinesterase, acetylthiocholine (EPMR=320) and acetylselenocholine (EPMR=220), are antiplanar at τ_2 and τ_3 both in crystals⁴² and in solution⁴³. It is known³⁰ to be sterically possible for these compounds to adopt a conformation with τ_2 equal to approximately $+120^\circ$, and we conclude that a small population of molecules with this conformation is responsible for activity. Activity is reduced, however, because τ_2 anticlinal is not the most stable conformation, the sulphur atom is larger than oxygen which moves methyl group C7 further away from the nitrogen atom⁴⁴, and τ_3 is at 129° and 124° respectively, outside the allowed range for potent muscarinic activity.

The structure of the weakly active muscarinic agonist (–)-R-3-acetoxy-quinuclidine methiodide (EPMR=600)³⁵ has been analysed (R. W. B. and P. J. P., work to be published). The $\text{N}^+-\text{C}-\text{C}-\text{O}$ torsion angle τ_2 is fixed at approximately $+107^\circ$ by the rigidity of the twisted (by 12°) quinuclidine ring structure and τ_3 at $+76^\circ$. This (τ_3) observed torsion angle explains the low activity of this substance.

The most potent isomer and enantiomer of 1-trimethylammonium 2-acetoxy-decalin is the (+)-axial-axial-*trans* derivative (EPMR=1,700)²⁴. This substance was expected to be a potent muscarinic agonist, but is weakly active. In the crystal structure³⁰ of this fairly rigid molecule, τ_2 is observed at $+147^\circ$ and τ_3 at approximately -90° . The weak activity of the substance is primarily a result of τ_3 being 60° out of the allowed range.

The inactive compound *threo*-acetyl- α,β -dimethylcholine²⁴ (EPMR=2,800) in crystals of the iodide²³ has torsion angles $\tau_2 = +143^\circ$ and $\tau_3 = -95^\circ$. This compound is inactive because its observed conformation with respect to τ_3 is different from that required for muscarinic activity. Lactoylcholine, although a nicotinic agonist, is relatively inactive as a muscarinic agonist⁴⁵. In crystals of the iodide⁴⁶, it has the conformation of a typical muscarinic agonist with $\tau_2 = +85^\circ$ and $\tau_3 = +157^\circ$, but the substituted hydroxy and methyl groups of lactic acid interfere with the interaction between the methyl group C7 and the receptor³⁴ and account for its inactivity. Although *trans*-ACTM is a potent muscarinic agonist, *cis*-ACTM is inactive²⁸. The τ_2 angle of *cis*-ACTM is necessarily nearly 0° because of the rigid cyclopropane ring, which is inconsistent with that of potent muscarinic agonists.

Acetylcholine and Other Substances

Our conclusions are that for potent muscarinic activity, in terms of Fig. 1, $\tau_1 = 180^\circ$ to C3 , τ_2 is in the range $+73^\circ$ to $+137^\circ$, $\tau_3 = 180 \pm 35^\circ$ and $\tau_4 = \text{either } 180^\circ \text{ or } -137^\circ$. The interatomic distances are: $\text{N}^+-\text{O1} = 320$; $\text{N}^+-\text{C6} = 450$; and $\text{N}^+-\text{C7} = 540$ pm. All known stereospecific muscarinic agonists have τ_2 positive in the range $+73^\circ$ to $+137^\circ$.

The conformation of acetylcholine relevant to the muscarinic receptor (Fig. 1) is that in which the trimethylammonium group is staggered, with: $\tau_1 = 180^\circ$; τ_2 somewhat greater than positive synclinal, approximately $+85^\circ$; τ_3 approximately $+150^\circ$ (the usual value for esters of primary alcohols, see ref. 47 and P. J. P., in preparation); and $\tau_4 = 180^\circ$, the universally observed antiplanar ester group. The hydrogen atoms of the trimethylammonium groups have been universally observed in several of the X-ray diffraction studies described and a neutron diffraction study⁴⁸ to be in a staggered conformation (one $\text{H}-\text{C}-\text{N}-\text{C} = 180^\circ$). One hydrogen atom of methyl group C7 is always observed to be synplanar to the carbonyl oxygen atom O2 in esters and presumably the conformation of ACh relevant to receptors.

The conformational rules for potent muscarinic activity presented here can be applied to many compounds reported to be muscarinically active, which have unknown conformations³. These include, for example, γ -crotonic betaine, F2581 (2-methyl-1,3-dioxan-5-yl-trimethylammonium), choline ethyl ether, *n*-pentyltrimethylammonium and Scheuler's reversed ACh [methyl-(β -trimethylammonium)propionate]¹.

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Common Genetic Alterations of RNA Tumour Viruses grown in Human Cells

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Growth of serologically distinct murine RNA tumour viruses in human cells results in the acquisition of common viral surface antigens that are genetically stable. The altered viruses are able to replicate much more efficiently than the parent viruses in human cells. These findings can best be interpreted on the basis of recombination of viral and human genetic information.

It is well established that mammalian¹⁻³ as well as avian^{4,5} RNA-containing sarcoma viruses can transform human cells. What, if any, alteration in the virus might occur in such circumstances has not yet been examined. In our laboratory, a human fibroblast culture was transformed by a focus-purified virus strain of Kirsten murine sarcoma virus KiMSV (KiMuLV)¹, and propagated for many cell generations during which it maintained its altered morphology. Although the virus released was unchanged after a few cell generations, we found that after prolonged passage of the transformed cells, the host range of the virus became markedly altered. We have investigated whether this alteration represented a genetic change in the virus or was due simply to modification of the viral coat in the process of budding from the human cell membrane. Our findings indicate that prolonged growth of murine tumour viruses in human cells leads to a genetically stable change in host range and to the development of new viral surface antigens. These seem to result from the acquisition by the viruses of human genetic information.

Cells were grown in Dulbecco's modification of Eagle's

medium. The continuous mouse cell lines, BALB/3T3 (ref. 6) and NIH/3T3 (ref. 7), and the normal rat kidney (NRK) cell line⁸ have been described before. A human fibroblast strain was derived from a skin biopsy of a normal person¹. The viruses used included a focus-purified stock of KiMSV (KiMuLV) which consists of two viruses, a transforming virus and a non-focus forming helper murine leukaemia virus, KiMuLV (S. A. A. and C. W. Weaver, unpublished). The transforming virus was titrated by a focus-forming assay⁹, while the helper virus associated with it was assayed by the XC plaque test¹⁰, a quantitative method for titration of MuLV. The complement fixation assay for viruses of the murine sarcoma-leukaemia complex has been reported previously¹¹. Rauscher leukaemia virus (R-MuLV) was a gift of Dr F. Rauscher, NIH. R-MuLV, which had been adapted to growth in human cells¹² and is termed H-R-MuLV, was a gift of Dr D. Ablashi, NIH. Leukaemia virus pseudotypes of KiMSV were obtained by co-cultivation of MuLV-releasing cultures with non-producer mouse or rat cell lines. These lines have been shown to contain the KiMSV genome in a non-replicating state and required the presence of MuLV for rescue of an infectious sarcoma virus (ref. 9 and unpublished results of S. A. A. and C. W. Weaver).

Neutralizing antisera were prepared in rabbits by Dr W. Parks, NIH, as previously described¹³. Rabbit antiserum to H-R-MuLV was supplied by Dr Ablashi. Neutralization tests were performed by a focus reduction method. About 100 focus-forming units (f.f.u.) of a sarcoma virus stock were exposed to the appropriate dilution of neutralizing antiserum for 30 min at 37° C and then assayed for residual focus-forming activity.

Alteration of Host Range

Earlier studies, which demonstrated that KiMSV (KiMuLV) was capable of transforming human cells, had revealed no

alteration in the host range of the virus produced during about 4 weeks after cellular transformation¹. After continued cell passage on a weekly transfer schedule for several more weeks, the sarcoma virus released lost its ability to transform mouse NIH/3T3 cells (Table 1). Furthermore, it became relatively more efficient at growing in human cells. It was now able to induce transformed foci almost as efficiently in human fibroblasts as in NRK cells. This change in host range was unexpected, for when KiMSV (KiMuLV) was grown in either NRK or NIH/3T3 cells for long periods, it showed no such alteration. The change in host range seemed unlikely to be due to host adaptation (a non-genetic change in the viral coat such as that which is known to occur with myxoviruses¹⁴) because of the length of time it took to become manifest.

Table 1 Altered Host Range of KiMSV (KiMuLV) after Growth in Human Cells

Virus grown in	Sarcoma virus titre (f.f.u./ml.) when assayed in *		
	NIH/3T3	NRK	Human fibroblasts
NRK	10 ^{5.5}	10 ^{5.6}	10 ^{2.8}
Human cells (four cell transfers)	10 ^{3.3}	10 ^{3.6}	10 ^{1.2}
Human cells (sixteen cell transfers)	Negative †	10 ^{2.8}	10 ^{2.7}
NRK (third passage of virus from human cells at sixteenth transfer)	Negative	10 ^{5.2}	10 ^{4.5}

* Cells were inoculated at 2×10^5 cells per Petri dish 24 h before infection. After pretreatment for 1 h with diethylaminoethyl-dextran (25 µg/ml.), cultures were inoculated with appropriate dilutions of virus and assayed for focus formation at 7 days (NIH/3T3 and NRK cells) or 12 days (human fibroblasts). Focus formation exhibited "one hit" kinetics under the experimental conditions¹⁰.

† Negative means no foci in a total of four Petri dishes inoculated with undiluted virus.

It was possible to test whether the striking change in host range of the human cell-grown KiMSV (KiMuLV) was due to host adaptation or to a genetic alteration of the virus stock. NRK cells were infected with the altered virus, and the virus was passaged twice more in NRK cells during the next 2 weeks on the grounds that growth back in NRK cells should eliminate any alteration that was not genetically stable. As Table 1 shows, growth of the virus in NRK cells resulted in a 100-fold increase in MSV titre when assayed in either human or NRK cells. But in spite of this increased titre, the virus was still unable to transform mouse NIH/3T3 cells. I therefore concluded that the virus had undergone a genetic modification during its growth in human cells.

The loss of ability of KiMSV (KiMuLV) to transform mouse cells could be due to a change in the genetic information of the sarcoma or of the leukaemia virus which provides certain functions required for sarcoma virus replication⁹. To test whether the helper leukaemia virus associated with the sarcoma virus was also altered, NIH/3T3 cultures were infected with the altered virus stock after it had first been grown for several viral generations in NRK cells. There was no XC plaque formation nor was there evidence of induction of complement-fixing antigens of the murine-leukaemia-sarcoma complex in such cultures. It should be noted that NIH/3T3 cells are sensitive to XC plaque formation and to the induction of a murine leukaemia viral complement-fixing antigen in response to KiMuLV. Whatever genetic change occurred during passage in human cells, therefore, led to an alteration in the

host range of both the sarcoma and leukaemia viruses in the KiMSV (KiMuLV) stock. For the present purposes the human cell-modified virus will be termed KiMSV (H-KiMuLV).

Genetic Alteration of R-MuLV

Wright and Korol¹² have reported infection of human cells with R-MuLV. This virus could be transmitted to other human cells, but its host range was not extensively investigated. In view of the altered properties of KiMSV (H-KiMuLV), I decided to examine carefully the host range of H-R-MuLV. An H-R-MuLV pseudotype of KiMSV was produced by pseudotype rescue from a non-producer KiMSV-transformed NRK line. The rescued virus was then inoculated onto human, NIH/3T3, and NRK cells. The rescued focus-forming virus, KiMSV (H-R-MuLV), was able to transform human and NRK cells with almost equal efficiency but was unable to induce focus formation in mouse cells (Table 2). In contrast, an R-MuLV pseudotype of KiMSV, which was obtained by pseudotype rescue from the same non-producer line, grew with equal efficiency in the mouse and rat cells, but was extremely inefficient at transforming human cells. These results indicated a common pattern of host range alteration for two different murine tumour viruses grown in human cells. Each virus had lost its capacity to infect mouse cells while becoming much more efficient at growth in human cells.

Table 2 Host Range of R-MuLV and H-R-MuLV Pseudotypes of KiMSV

Virus	Sarcoma virus titre (f.f.u./ml.) when assayed in *		
	NIH/3T3	NRK	Human fibroblasts
KiMSV (R-MuLV)	10 ^{5.7}	10 ^{5.2}	Negative
KiMSV (H-R-MuLV)	Negative †	10 ^{4.2}	10 ^{3.5}

* Focus forming assays were performed as described in Table 1.

† Negative means no foci in a total of four Petri dishes inoculated with undiluted virus.

Although H-R-MuLV and KiMSV (H-KiMuLV) had each lost their infectivity for mouse cells, they did retain their murine character as defined by the persistence of murine group specific (gs) antigens. Human or NRK cells infected with either of the altered viruses developed murine viral group specific antigens detectable by complement fixation.

Surface Antigens of the Altered Tumour Viruses

By the use of neutralizing antisera, viruses of the murine leukaemia-sarcoma complex can be classified in one of two major serological groups: Friend-Rauscher-Moloney and Gross-type. R-MuLV is a member of the former while KiMuLV is a member of the latter. Rabbit antisera were obtained against R-MuLV, KiMSV (KiMuLV), and each of the genetically altered viruses. It has been shown that a leukaemia virus pseudotype of MSV has the same neutralization characteristics as the leukaemia virus itself^{9,15}. Neutralization assays were performed by the focus reduction method, and all virus preparations were standardized by growth for three viral passages in NRK cells.

The R-MuLV and KiMuLV pseudotypes of KiMSV were clearly distinguishable by use of antisera made against each virus (Table 3). Neither KiMSV (R-MuLV) nor KiMSV

(H-R-MuLV) was inhibited by antiserum which completely neutralized KiMSV (KiMuLV) and neither KiMSV (KiMuLV) nor KiMSV (H-KiMuLV) was affected by antiserum to R-MuLV. Each of the genetically altered viruses was neutralized by antiserum to its parent virus. These latter results indicated that each human-modified virus had retained at least some surface antigens of its original virus stock.

Table 3 Neutralization of MuLV Pseudotypes of KiMSV by Viral Antisera

Virus	% reduction in focus formation after treatment with antisera against			
	R-MuLV	KiMSV (KiMuLV)	H-R-MuLV	KiMSV (H-KiMuLV)
KiMSV (R-MuLV)	100	0	0	0
KiMSV (H-R-MuLV)	95	0	100	100
KiMSV (KiMuLV)	0	100	0	0
KiMSV (H-KiMuLV)	0	100	100	100

Neutralization tests were performed by the focus reduction method. About 100 f.f.u. of each sarcoma pseudotype were exposed to neutralizing antiserum for 30 min at 37° C and then assayed on diethylaminoethyl-dextran pretreated NRK cells. The number of MSV foci was scored at 7 days. For the production of viral antisera, R-MuLV was grown in mouse cells, H-R-MuLV in human cells, and both KiMSV (KiMuLV) and KiMSV (H-KiMuLV) in NRK cells. Each antiserum was used at a final dilution of 1:60.

Table 4 Neutralization Titres of Antisera against Pseudotypes of KiMSV

Virus	Neutralization titre* of antiserum against:			
	R-MuLV	KiMSV (KiMuLV)	H-R-MuLV	KiMSV (H-KiMuLV)
KiMSV (R-MuLV)	600	< 20	< 20	< 20
KiMSV (H-R-MuLV)	100	< 20	300	300
KiMSV (KiMuLV)	< 20	200	< 20	< 20
KiMSV (H-KiMuLV)	< 20	200	300	300

* Reciprocal of highest antiserum dilution giving 67% or greater reduction in the number of MSV foci when tested against about 100 f.f.u. of the appropriate MuLV pseudotype of KiMSV.

Of most interest were the results of neutralization studies using antisera to each of the genetically altered viruses. Antiserum to H-R-MuLV was a potent inhibitor of focus-formation by both human-modified viruses (Table 3). Similarly, antiserum to KiMSV (H-KiMuLV) completely neutralized both viruses. In contrast, neither antiserum affected the parent virus strains. Table 4 shows the actual end point neutralization titres of the different antisera confirming the findings in Table 3. These results imply the existence of common surface antigens in the genetically altered viruses that are not detectable in the original virus stocks.

Possible Mechanisms for Genetic Alteration

These studies clearly demonstrate that mammalian RNA tumour viruses can be genetically altered during growth in human cells. Previously, avian tumour viruses have been observed to show changes in host range and/or serological characteristics after passage in a foreign species either *in vivo*¹⁶⁻¹⁸ or *in vitro*¹⁹. Genetic alteration of the virus in such

circumstances has been suggested¹⁹. The alteration in the murine tumour viruses reported here must have resulted from one of two processes: mutation during growth and selection in human cells or modification of the virus by recombination with human genetic information. An explanation of the results based on selection in human cells of a minority population initially present in the KiMSV (KiMuLV) stock is unlikely because it was focus-purified immediately before use.

Of the two hypotheses, the evidence strongly favours recombination. It seems highly unlikely that random mutations would occur in two murine tumour viruses grown independently so as to affect their host ranges in an identical manner. Furthermore, that each should develop surface antigens that are distinct from those of their parent viruses yet common to each other is very difficult to fit into a mutation-selection model. There is biochemical evidence for some homology between host cell and RNA tumour virus genetic information (ref. 20 and unpublished results of L. D. Gelb, S. A. A., and M. A. Martin). The biological significance of these findings is not clear. Whether such biochemical techniques will be useful in detecting recombinant human genetic information in the human-altered murine viruses is being tested.

The mechanism by which recombination between viral and host genetic information might occur is still a subject for speculation. The discovery of an RNA-dependent DNA polymerase in RNA tumour viruses^{21,22} clearly supports the hypothesis that these viruses replicate and possibly integrate into cells by means of a DNA intermediate²³. The most exciting possibility is that recombination has occurred with a latent C-type human virus which itself may exist in an integrated state. The new viral surface antigens of the murine viruses described here would then reflect genetic information of this latent virus. These antigens could be very useful markers with which to search for serological evidence of a viral aetiology for human cancer.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Possibility of a 44 Å Line Component in the Diffuse Cosmic X-ray Flux

SEVERAL investigators have measured the diffuse cosmic X-ray intensity in the 1/4 keV band by means of rocket-borne gas proportional counters¹⁻⁵. Intensities derived from these measurements have used the assumption that the incident X-rays are continuously distributed in energy over the band-passes of the detectors, and the results disagree. We have therefore reinterpreted existing data, giving consideration to the possibility of line emission (possibly carbon K α) being superposed on the cosmic X-ray continuum. With this generalization, the discordance of the reported fluxes is substantially reduced. This may be simply an artefact of the existing data, but the possibility that it reflects the existence of a line in the diffuse X-ray background is sufficiently important for us to urge that new data be examined for this possibility as they become available.

In the $\frac{1}{4}$ keV band, the efficiency of a detector is equal to the transmission of its window, $T(E)$. We shall represent the continuous X-ray spectrum as a power law over the $\frac{1}{4}$ keV band: $I(E) = I_0 \cdot (E/E_k)^{-2}$ photons per (cm² s ster keV), where E_k is the carbon K absorption edge energy (284 eV). The index of -2 is representative of the values (-1 to -3.3) used by the authors cited. In the presence of this continuum and also line radiation of strength J photons per (cm² s ster), a detector would measure a count rate per unit area and solid angle given by

$$F = \int_0^{E_k} I(E) T(E) dE + TJ$$

$$= BI_0 + TJ \quad (1)$$

T represents the transmission for the line radiation which we shall take to be at 277 eV; B represents the (weighted) integrated transmission bandpass

$$\int_0^{E_k} T(E) \cdot (E/E_k)^{-2}$$

Because such integrands are sharply peaked at E_k the band-passes are not strongly sensitive to the assumed spectral index.

Existing measurements have been interpreted in terms of continuum radiation, so that apparent intensities I' have in effect been derived by division of observed count rates by appropriate values of B :

$$I' \equiv F/B = I_0 + \frac{T}{B} J$$

Because (T/B) is a function of window thickness, reported intensities I' will exhibit a similar dependence if line emission is significant.

In Fig. 1, I' is plotted against the corresponding value T/B . Because of the unknown cloud structure of the galactic medium, no systematic correction to I' for interstellar absorption has been attempted here; rather, high galactic latitude data have been used which, where possible, incorporate an extrapolation to $N_H = 0$ based on the small, empirical N_H dependence. Fig. 1 demonstrates that these available data are not consistent with a pure continuum spectrum, for which I' would be independent of T/B . Instead, a marked upward trend can be discerned with slope $J \cong 25$ photons per (cm² s ster). The I' -intercept of this trend gives the residual continuum intensity I_0 which, for these data, is consistent with zero.

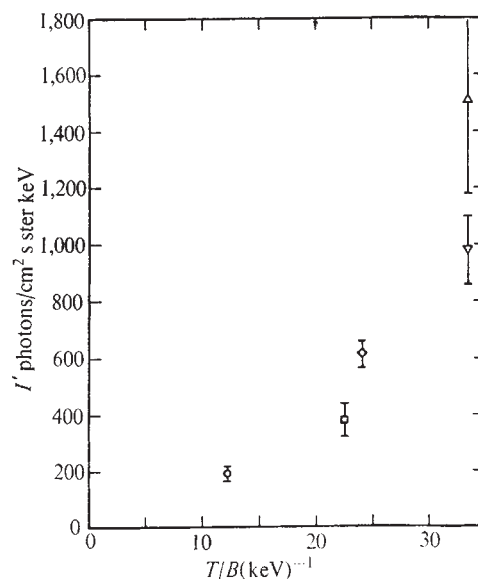


Fig. 1 Reported values of the apparent continuum intensity I' as a function of the transmission/bandwidth ratio, T/B , with which each measurement was made. Δ , ref. 4; $\square$, ref. 1; $\circ$, ref. 2; $\diamond$, ref. 3; ∇ , ref. 5.

It is important to determine that systematic effects other than line radiation will not mimic the apparent trend of I' with T/B . One such cause might be a continuum, the energy spectrum of which is substantially different from E^{-2} , in which case the computed values T/B used in plotting Fig. 1 are only approximately correct. Detailed investigation shows, however, that large positive exponents would be required to cause the trend observed, and such spectra can be ruled out on the basis of numerous measurements above $\frac{1}{4}$ keV.

We have also considered two processes whereby a spurious $\frac{1}{4}$ keV signal could result from absorption of a more energetic photon in the counter window: (a) detection of K-fluorescence radiation emitted by carbon atoms in the window, and (b) detection of photoelectrons ejected from the window. The quantitative difficulty with the first possibility is the low fluores-

cent yield of carbon (10^{-3}); expected count rates are of the order of 0.01 count per ($\text{cm}^2 \text{ s ster}$) for $600 \mu\text{g}/\text{cm}^2$ windows. Similarly, the short range of kilovolt electrons ($1\text{--}10 \mu\text{g}/\text{cm}^2$) restricts detection to those produced in the rearmost few micrograms/ cm^2 of the window; expected count rates for process (b) are less than 0.1 count per ($\text{cm}^2 \text{ s ster}$). These processes thus seem to be insufficient to explain the observed $\frac{1}{2}$ keV count rates, which range from 1 to 10 counts per ($\text{cm}^2 \text{ s ster}$).

As independent support for the hypothesis of line emission, we cite the finding of Bunner *et al.*² that the soft X-ray fluxes measured during one rocket flight with detectors having 300 and $600 \mu\text{g}/\text{cm}^2$ Mylar-equivalent windows were in the ratio 2.7 ± 0.3 , a value outside the range expected for continuum radiation but appropriate to a spectrum dominated by the 277 eV line.

If the observed $\frac{1}{2}$ keV radiation is in fact line emission, it is important to determine the location of the emitting matter. Galactic absorption of the radiation has been well established; thus the source must predominantly lie beyond the Galaxy. Since a dispersed source of line emission integrated over a cosmologically significant path length would be redshifted into a continuum and since data at our disposal suggest that the line is no broader than about $5 \text{ \AA}$, it follows that the hypothetical diffuse source must be localized within $z=0.1$.

A possible source of line emission is interstellar or intergalactic matter which contains at least a small proportion of carbon. Carbon K shells photoelectrically absorb cosmic X-rays at a rate

$$\int_{\frac{1}{2}}^{\infty} \sigma(E) \cdot 4\pi I(E) dE$$

and a fraction W_K of these absorptions is followed by fluorescent X-ray emission. The resulting sky brightness arising from this process is then

$$J = \frac{W_K N_C}{4\pi} \int_{\frac{1}{2}}^{\infty} \sigma(E) \cdot 4\pi I(E) dE$$

where N_C is the column density of neutral carbon atoms (carbon ions have too high a fluorescence energy to contribute to the observed flux). Matter associated with the galactic halo might have $N_C/N_H = 10^{-3}$ and $N_H = 2 \times 10^{20}$ so that $J = 0.01$ photon per ($\text{cm}^2 \text{ s ster}$). If instead a carbon abundance of 10^{-3} occurs in a cosmological model having $n_H = 2 \times 10^{-5}$, a path length of $z=0.1$ would contribute a brightness of $J=1$ photon ($\text{cm}^2 \text{ s ster}$), which is still inadequate by a factor of twenty.

We conclude that the reported intensities of the low energy diffuse X-ray background flux indicate a systematic dependence upon window thickness. This dependence is not easily explained in terms of continuum radiation or as an instrumental effect but can be ascribed to $44 \text{ \AA}$ line emission with an intensity of 25 photons per ($\text{cm}^2 \text{ s ster}$).

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Direct Observation of Solvation of the Electron in Liquid Alcohols by Pulse Radiolysis

THE solvated electron (e_s^-) is an important intermediate in the radiation chemistry of liquids, and being the simplest of chemically reactive species, its properties are of some interest¹. The interaction of the electron with its immediate environment or solvation shell is important in the formulation of models for e_s^- , and direct observation of the change from a "dry" to a "solvated" electron is likely to provide information for such models. Also, because the dry electron diffuses roughly three orders of magnitude faster than e_s^- , the time required for solvation may have important chemical consequences in the effects of ionizing radiation on polar liquids². We have recently obtained direct evidence for the recombination of electrons with their geminate cations before solvation can occur (unpublished results).

The solvation process can be examined by following the appearance of the optical absorption band of e_s^- ($\lambda_{\text{max}} \approx 500\text{--}800 \text{ nm}$) which is due to its solvated state. We have now been able to do this for liquid alcohols at low temperature using nanosecond pulse radiolysis. We have irradiated alcohols in a variable-temperature cell which permits sample changing to avoid build-up of radiolysis products. Electron pulses (5–10 ns) from a 12 MeV linear accelerator gave doses of ~ 800 rads/pulse and absorptions were measured during and after the pulse, using equipment with a rise time of $\sim 1\text{--}2$ ns.

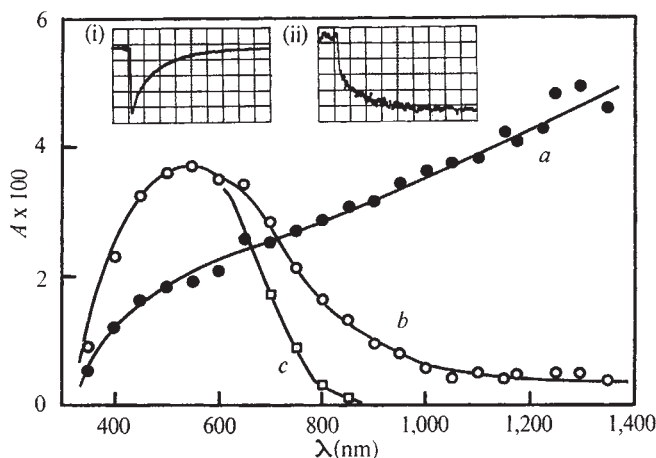


Fig. 1 Absorption spectra after a 5 ns, ~ 600 rads electron pulse in n-propanol at 152 K. a, At end of pulse; b, 0.2 μs after pulse; c, 1 μs after pulse. Insets: CRO traces of absorption changes on 50 ns/cm, (i) 1,300 nm, (ii) 500 nm.

We find that the optical absorptions due to e_s^- in liquid alcohols are time dependent as shown in Fig. 1 where three distinct changes are apparent. Initially there is a structureless absorption (curve a) rising steadily from $<350 \text{ nm}$ to $>1,350 \text{ nm}$ which is qualitatively similar to the absorption tail of the electron trapped in glassy hydrocarbons⁴ and may be attributed to a solvent environment which has not yet reacted to the presence of the charge. This absorption in the near infrared then decays and simultaneously (half life τ_s) the characteristic visible band of e_s^- grows in. But, even when the absorption at $\lambda > 1,100 \text{ nm}$ has completely decayed, the spectrum (curve b) is still not that which e_s^- is known³ to have in its equilibrium or "long time" state, in that there is much more absorption between ~ 600 and $1,100 \text{ nm}$. This red shoulder then decays to leave the equilibrium spectrum (curve c), but there is no simultaneous grow-in at $\lambda < 600 \text{ nm}$ as occurred in the initial stage. Subsequent, much slower changes are only in the intensity of absorption (the spectrum remains the same).

and they are due to spur recombination of e_s^- with the geminate cations, followed by the homogeneous reaction of the free, extra-spur electrons with the solvent. The first half lives of these spur recombinations are orders of magnitude greater than τ_s being $\sim 0.1 \mu\text{s}$ in CH_3OH at 182 K, $1 \mu\text{s}$ in $\text{C}_2\text{H}_5\text{OH}$ at 166 K, $6 \mu\text{s}$ in $n\text{-C}_3\text{H}_7\text{OH}$ at 152 K, $3 \mu\text{s}$ in $(\text{CH}_3)_2\text{CHOH}$ at 186 K and $4 \mu\text{s}$ in $n\text{-C}_4\text{H}_9\text{OH}$ at 184 K. The final reactions of e_s^- with the solvent have $t_1 \geq 1 \text{ ms}$.

Although all these alcohols show the same qualitative behaviour as illustrated in Fig. 1, the times during which the initial spectral changes occur are markedly affected by temperature and vary between alcohols, being most rapid in the alcohol with shortest dielectric relaxation time. Table 1 shows the half lives τ_s of the initial change, that is, the decay of the near infrared absorption to produce the visible absorption.

Electron trapping in glassy solids at 77 K has been interpreted in terms of the dry electron diffusing to preformed traps or defects, because the concentration of electrons in several glasses goes through a maximum with increasing dose^{5,6}. Moreover, the dielectric relaxation times of alcohol glasses are estimated to be $10^{50}\text{--}10^{100} \text{ s}$ at 77 K. But spectral changes qualitatively similar to ours for liquids have been observed in glassy ethanol at 77 K by Richards and Thomas⁷. The changes are considerably slower in the glass (for example, the change to curve *b* of Fig. 1 takes $\sim 4 \mu\text{s}$). If this is due to solvent relaxation or the electron "digging its own hole" as has been suggested⁷, the presence of the electron must have an enormously greater effect on the relaxation time at 77 K than at $\sim 180 \text{ K}$. Moreover, changes which seem more likely to be normal solvent relaxation at low temperature occur over minutes at 90–100 K in γ -irradiated *n*-propanol glass⁸. A more likely possibility at 77 K is that electron thermalization during trapping produces local heating of the matrix, a process which would also explain the low yields of trapped electrons with high LET radiation⁹.

Three different dispersion regions have been observed in liquid alcohols at $\sim 300 \text{ K}$ ¹⁰ and $\sim 180 \text{ K}$ ¹¹. At $\sim 300 \text{ K}$ these have been attributed to the breaking up of hydrogen bonded clusters (relaxation time τ_1) and to the more rapid rotation of either monomeric molecules or hydroxyl groups (τ_2 and τ_3 respectively), although because few monomeric alcohol molecules are present¹² at low temperature τ_2 may not be attributable to the same process.

Table 1 Relaxation Times in Alcohols*

Alcohol	<i>T</i> (K)	$\tau_s \dagger$	τ_1'	τ_2'	τ_3'
CH_3OH	182	≤ 1	0.3	~ 0.01	< 0.01
$\text{C}_2\text{H}_5\text{OH}$	166	3	5	0.03	~ 0.01
$n\text{-C}_3\text{H}_7\text{OH}$	176	5	33	1.7	0.3
	152	60	750	33	2.7
$(\text{CH}_3)_2\text{CHOH}$	186	6	35	~ 1	~ 0.1
$n\text{-C}_4\text{H}_9\text{OH}$	184	4	40	0.8	~ 0.1

* All times are given in nanoseconds.

† Our measurements.

Table 1 shows that the solvation times τ_s are within an order of magnitude of the dielectric relaxation time at constant charge² $\tau_1' (= \tau_1 \cdot \epsilon_\infty/\epsilon_s)$ which we have estimated from the literature^{10,11}. Bronskill, Wolff and Hunt¹² estimated $\tau_s \approx 25 \text{ ps}$ in these alcohols at 293 K and from our measurements at $\sim 180 \text{ K}$ we estimate an activation energy for solvation of the electron of $15\text{--}20 \text{ kJ mol}^{-1}$, which is similar to that found for τ_1 and that expected for hydrogen bond rupture.

An interesting observation in this and the glass work⁷ is that $\sim 50\%$ of the equilibrium absorption at λ_{max} is already present immediately after the pulse. If absorption at λ_{max} indicates the existence of deep traps, then this suggests that capture of dry electrons in pre-existing traps occurs as well as solvent relaxation trapping. Spectral changes between 0.2 and $1 \mu\text{s}$ in Fig. 1 are probably not due to solvent relaxation, for there is no parallel increase in absorption elsewhere in the

spectrum. A possible explanation for these changes is that a small fraction of the electrons are "damp" rather than fully solvated because of the proximity of the positive ion and are disappearing in a spur recombination process with a rate intermediate between the "dry" and fully solvated species.

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Silver-108m in Biota and Sediments at Bikini and Eniwetok Atolls

THE occurrence¹ of the long lived silver radionuclide $^{108\text{m}}\text{Ag}$ ($T_{1/2} > 100 \text{ yr}$) in biota from the Pacific Ocean has been held to suggest that the silver radionuclide ratio $^{110\text{m}}\text{Ag}/^{108\text{m}}\text{Ag}$ may be useful as a tracer of environmental processes. It also appears¹ that large amounts of $^{110\text{m}}\text{Ag}$ and $^{108\text{m}}\text{Ag}$ were not produced during the 1958 test series or earlier, and that the production activity ratio $^{110\text{m}}\text{Ag}/^{108\text{m}}\text{Ag}$ was 162, a ratio derived solely from the thermal neutron activation of stable silver. We consider that their observations require reconsideration.

We have found $^{108\text{m}}\text{Ag}$ in biota and sediments collected at previous nuclear testing sites in the Pacific. We first detected the radionuclide in a composite sample of the hepatopancreases of spiny lobsters collected at Bikini Atoll in 1969, and have since measured its concentration in several samples from Bikini and Eniwetok Atolls. Gamma-ray spectra were made using both NaI(Tl) and a solid state Ge(Li) detector system. The resolution of the Ge(Li) detection system permitted precise identification of the radionuclide photopeaks and the NaI(Tl) crystal systems were used quantitatively to measure the $^{108\text{m}}\text{Ag}$. More specific information was obtained by chemically isolating the $^{108\text{m}}\text{Ag}$ using a solvent extraction technique² by means of which the silver radionuclides are effectively separated from a number of other radio elements, especially ^{125}Sb , ^{207}Bi , ^{60}Co , ^{54}Mn , ^{102}Ru and $^{230,232}\text{Th}$.

Comparison (Table 1) of the concentrations of $^{108\text{m}}\text{Ag}$ in the hepatopancreas of spiny lobsters taken from Bikini with that reported in ref. 1 shows that the Bikini specimens contain 2 to 3 times more $^{108\text{m}}\text{Ag}$ than those from Eniwetok Atoll. By contrast, the concentration of $^{108\text{m}}\text{Ag}$ in the composite sample from Eniwetok is less than that in the Guadalupe lobster specimen. The last test series at Bikini and Eniwetok Atolls occurred in 1958, so that the time between the cessation of

tests and the collection of the specimens ranges from 6 to 12 yr. It is reasonable to conclude that higher concentrations of both ^{108}mAg and ^{110}mAg were present at earlier times. Indeed, Seymour³ has reported a ^{110}mAg concentration of approximately 100 d.p.m./g of wet tissue for the hepatopancreas of a spiny lobster collected at Guam in November 1959, and although ^{108}mAg was not determined in the sample, it is significant that this activity was observed in biota collected 1,200 miles downstream of the test site as early as 1959.

Table 1 Concentrations of ^{108}mAg in Samples from Bikini and Eniwetok Atolls

Location	Samples	Date of collection	Concentration of ^{108}mAg (d.p.m./g dry wt)
Eniwetok Atoll	Spiny lobster (20)* (hepatopancreas)	August 1964	0.20 ± 0.04 †
Bikini Atoll	Spiny lobster (3) (hepatopancreas)	June 1969	1.1 ± 0.3
Bikini Atoll	Spiny lobster (9) (hepatopancreas)	June 1970	0.75 ± 0.15
Bikini Atoll	Crater sediment	July 1969	0.09 ± 0.05

* No. in parentheses signifies the number of individuals comprising the composite sample analysed. Species: *Panulirus* sp.

† Errors represent the 67% confidence level of the count rate measurements.

The sediment (Table 1) was taken from the site of a large thermonuclear detonation in 1954 (Bravo Crater), the crater of which is exposed to both lagoon and sea water and which is therefore a source of ^{108}mAg for the biota of the area (^{110}mAg having largely decayed between 1954 and 1969). Thermonuclear tests at other sites in the atoll have also produced silver radionuclides. The last test at Bikini was in 1958, yet we have measured ^{110}mAg concentration of 5.2 ± 0.06 d.p.m./g of soil on Eninman Islet at the atoll and thus the amount of ^{108}mAg present in the Pacific Ocean and elsewhere as a result of pre-1959 testing may be significant.

The second observation about the initial production activity ratio of $^{110}\text{mAg}/^{108}\text{mAg}$ in ref. 1 may be conservative in its estimate of ^{108}mAg production. As we have said, the initial activity ratio of $^{110}\text{mAg}/^{108}\text{mAg}$ was assumed to be due only to thermal neutron activation of stable silver. There are other production mechanisms for these two radionuclides, the most notable being (n,2n) reactions on stable silver and (n,p) reactions on stable cadmium. The cadmium reaction is probably not important because the quantity of stable cadmium in nuclear devices would probably be kept to a minimum on account of the large thermal neutron cross-sections. The excitation functions for (n,p) reactions at atomic weights between 100–110 and neutron energies between 10–20 MeV (ref. 4) are small (1–100 millibarns) by contrast with the excitation functions for (n,2n) reactions at atomic weights > 100 and similar neutron energies, which are significantly higher (> 1 barn).

We have irradiated stable silver in a fast neutron beam at the University of Washington cyclotron [$^9\text{Be}(d,n)^{10}\text{B}$] and have produced easily measurable quantities of ^{106}mAg [$^{107}\text{Ag}(n,2n)^{106}\text{mAg}$] and ^{105}Ag [$^{107}\text{Ag}(n,3n)^{105}\text{Ag}$]; ^{108}mAg has not been found so far in the irradiated sample. The energy similarities of the gamma rays emitted by ^{106}mAg and the very low specific activity of ^{108}mAg will require several months decay time before the presence or absence of ^{108}mAg can be confirmed. The (n, γ) cross-sections for ^{107}Ag and ^{109}Ag (based on a radiative capture initial activity ratio of 162) seem, however, to be almost equal for $^{110}\text{mAg}/^{108}\text{mAg}$ and so the nuclear states of the two isotopes seem to be similar. It would be surprising if ^{108}mAg

production by (n,2n) reactions on ^{109}Ag were inconsequential, for (n,2n) reactions on ^{107}Ag have been shown experimentally to produce relatively large amounts of ^{106}mAg . Production pathways are important, because the type and design of a nuclear device can generate different ratios of fast and thermal neutron fluxes.

It is difficult to resolve our findings with the close agreement previously shown¹ between silver radionuclide activity ratios and the time of large scale nuclear testing. If it is possible for the silver radionuclides produced in 1961 and 1962 to remain in rather shallow isolated water masses for several years¹, then the same possibility must be extended to silver radionuclides produced before 1959. But the absence of definite knowledge of the rates at which silver is removed from the surface layers of the ocean makes it impossible to decide whether or not ^{108}mAg from pre-1961–62 testing confuses subsequent dating.

The determination of $^{110}\text{mAg}/^{108}\text{mAg}$ ratios in air filters would clarify the production activity ratios, and the analysis of undisturbed lichen samples collected annually since 1958 (and earlier) would elucidate the relative amounts of ^{108}mAg caused by pre-1959 tests and those in 1961–62. The potential usefulness of the $^{110}\text{mAg}/^{108}\text{mAg}$ ratio in dating is apparent but we feel that some caution should be exercised in their use for the description of natural processes until the outstanding problems are resolved.

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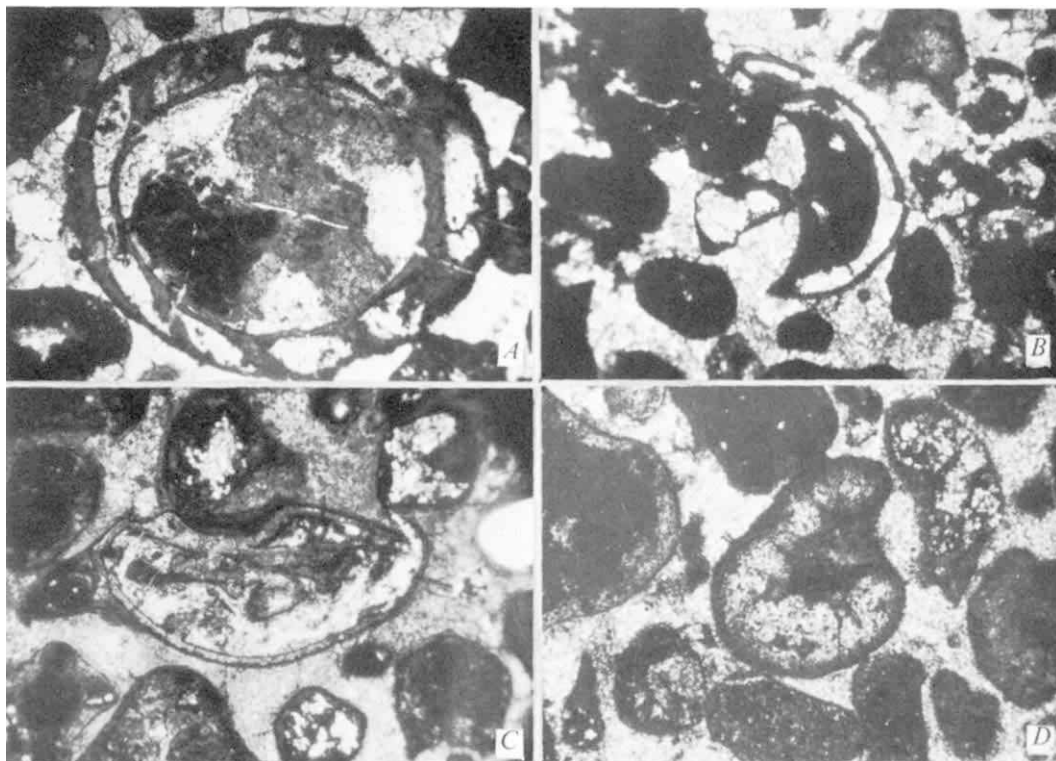
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Discovery of Pre-tertiary Fossils indigenous to the Lower Himalayan Basin

THE prolific abundance of fossils in the sediments of the Tethyan (or Tibetan) Himalayas and their reported absence in the pre-Tertiary sediments of the Lower Himalayas have been explained time and again by envisaging a highland¹, a geanticline² or a ridge³ between the two basins. This difference has also been explained by proposing a vast distance⁴ between the two basins, on the basis of palaeomagnetic data⁵. The use of palaeomagnetic data in the studies of global tectonics has, however, been convincingly challenged by Meyerhoff⁶, who further states that the present geographical relation of India with Asia has been constant since middle Proterozoic times. Whether or not the Himalayas originated as a consequence of continental drift⁷ remains undecided. But we can now provide evidence that the Lower Himalayas are not as barren of fossils as has been supposed⁸. Maybe there is no need to postulate a physical barrier between the Tethyan and the Lower Himalayan Basins. Recent discoveries of algal structures^{9,10} and coccolithophorids¹¹ from the Krols are also important in this context.

Fig. 1 A, B and C, photomicrographs of invertebrate fossil remains with skeletal structures, partly to well preserved ($\times 34$, $\times 34$, $\times 36$); D, photomicrograph of a microcoprolite with convolutions preserved on the concave side, suggesting the passage through the intestine of some organism ($\times 30$).



Invertebrate fossil remains and microcoprolites (Fig. 1) have been found for the first time from the pelletal phosphorite within the sequence of limestone-black shale-chert, exposed around Mussoorie. As this sequence represents the transition between the Krol formation (Permo-Triassic?) and the Lower Tal Series (Upper Triassic-L. Cretaceous?) in the Lower Himalayan zone, this find is of prime importance. It has improved the likelihood of finding further organic remains in the several black shale, chert sequences (often pyritiferous) intercalated within the Krol formation as well as in still older formations¹². The black colour in sediments is attributed to dispersed organic matter (maybe solid or liquid hydrocarbons) and the non-graphitic carbonaceous matter may represent degraded organisms living at that time¹³. The presence of phosphorite associated with such beds in the basal part of Krol A member¹⁴ (Permian?) is also significant in this context. There are published occurrences of microbiological remains even in black shale-chert and limestone sequences of known pre-Palaeozoic age¹³, and we think that such promising beds, distributed throughout in the Palaeozoic and Mesozoic formations of the Lower Himalayas, have been inadequately searched.

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BIOLOGICAL SCIENCES

Biochemical Model for the Biological Methylation of Mercury suggested from Methylation Studies *in vivo* with *Neurospora crassa*

SINCE the discovery of methyl mercury formation in lake sediments contaminated with inorganic or phenyl mercury¹, there has been a good deal of speculation about the mechanism of this synthesis. One process involving methyl-cobalamin (a B₁₂-derivative) was demonstrated in cell-free extracts of methanogenic bacteria². Vitamin B₁₂ is not known to be involved in the metabolism of *Neurospora*^{3,4}, and so we have investigated the biosynthesis of methyl mercury in this organism, where the pathway should be different.

Chemically synthesized methyl mercury has been found to be more toxic than inorganic mercury to *Neurospora* (unpublished results of L. Landner) as well as to many other organisms⁵. At a cursory glance, therefore, microorganisms seem to produce a more toxic substance from a less toxic one⁶. In view of the fact that the microorganisms concerned have been exposed to inorganic mercury long enough to evolve detoxification mechanisms, this seems strange.

We have studied the relationship between the resistance of *Neurospora* to inorganic mercury and its ability to produce methyl mercury. The finding that loci determining the resistance towards Hg²⁺ and the synthesis of methionine are closely associated in one complex gene in *Staphylococcus*⁷ suggested a possible relationship between methylation of mercury and methionine biosynthesis. The possibility that the methylation of mercury *in vivo* is achieved by means of a direct transmethylation involving methionine or S-adenosyl-methionine seems less likely on the evidence provided by preliminary experiments with *Neurospora* (unpublished results of A. Jernelöv and L. Landner and personal communication with S. Kitamura). In no case was an increased yield of methyl mercury found when L-methionine was added to the HgCl₂-containing medium. The addition of L-methionine to the medium even rendered the HgCl₂ more toxic to the fungus. Many strains do not tolerate 10 p.p.m. of Hg²⁺ in the presence of methionine, but do so in

its absence or in the presence of other methyl donors like choline or betaine.

To investigate the connexion between resistance to Hg^{2+} and methylation, a series of selection experiments was carried out, starting with a homokaryotic isolate of the wild type strain 74-OR8-1a, with a low tolerance to Hg^{2+} . The procedure was done in two steps: mutations were induced by X-ray irradiation (20,000 r) and the irradiated conidia were plated on Hg^{2+} -containing media in Petri dishes. Mutants tolerant to 200 p.p.m. of Hg^{2+} were collected. A preliminary screening test for the mercury-methylating capacity of the isolates from the dishes revealed that the isolates from the 200 p.p.m. dishes yielded more methyl mercury than the original strain ($0.05 > P > 0.02$). In a second series of experiments, the tolerance level was increased to 225 p.p.m. of Hg^{2+} . The highly tolerant isolates as well as nonselected strains were then cultivated in Erlenmeyer flasks containing liquid Fries minimal medium⁸ with Hg^{2+} and a thiol added at different concentrations. (In preliminary experiments addition of thiols enhanced the yield of methyl mercury in tolerant as well as nontolerant strains.) The mycelial pads were collected after they had grown for 4 weeks at 22° C, washed in distilled water, dried at room temperature and analysed for methyl mercury content by gas chromatography with E.C., a slight modification of the method of Westö⁹.

The results (Table 1) include an increase in yield of methyl mercury in the isolates tolerant of 225 p.p.m. of Hg^{2+} relative to the starting material. Table 2 also shows that the highly tolerant isolate (IV-1) has a much higher methylating efficiency than other strains. These results suggest that the methylation of mercury in *Neurospora* does indeed imply a detoxification of Hg^{2+} .

The relationship between yield of methyl mercury and presence of thiols in the medium, as suggested by Tables 1 and 2, was further studied, using less tolerant isolates. Table 3 shows that a concentration of DL-homocysteine in excess of the concentration of Hg^{2+} (on a molar basis) enabled the most efficient synthesis of methyl mercury. A large surplus of DL-homocysteine reduced the efficiency and a surplus of Hg^{2+} prevented growth. No such relationship was obtained when L-cysteine replaced DL-homocysteine, even though L-cysteine also stimulates mercury-methylation. Other thiols tested, such as mercaptoacetic acid, dimercaptopropanol and glutathione, did not cause an increase in methyl mercury concentration in the cells. Furthermore, when a large surplus of DL-homoserine

Table 1 Concentration of Methyl Mercury ($\pm$ s.e.) in Mycelia after 4 Weeks of Growth (ng Hg/g cell)

	DL-Homocysteine added		L-Cysteine added	
	40 p.p.m. Hg^{2+}	80 p.p.m. Hg^{2+}	40 p.p.m. Hg^{2+}	80 p.p.m. Hg^{2+}
Original strain	269 $\pm$ 55	106 $\pm$ 79	79 $\pm$ 19	694 $\pm$ 214
Strain tolerant to 225 p.p.m. Hg^{2+}	3,675 $\pm$ 862	98 $\pm$ 65	680 $\pm$ 288	5,125 $\pm$ 1,209

Table 2 Concentration of Methyl Mercury ($\pm$ s.e.) in Mycelia after 4 Weeks of Growth (ng Hg/g cells)

Strain	DL-Homocysteine added		L-Cysteine added	
	40 p.p.m. Hg^{2+}	80 p.p.m. Hg^{2+}	40 p.p.m. Hg^{2+}	80 p.p.m. Hg^{2+}
IV-1 selected	3,675 $\pm$ 862	98 $\pm$ 65	680 $\pm$ 288	5,125 $\pm$ 1,209
V-2 selected	423 $\pm$ 24	43 $\pm$ 11	155 $\pm$ 43	743 $\pm$ 213
74OR8-1a unselected	176 $\pm$ 66	18 $\pm$ 4	109 $\pm$ 22	933 $\pm$ 264
Fiji unselected	30 $\pm$ 12	15 $\pm$ 8	28 $\pm$ 5	230 $\pm$ 26
Costa Rica unselected	59 $\pm$ 4	31 $\pm$ 5	54 $\pm$ 12	205 $\pm$ 19

Table 3 Concentrations and Total Amounts of Methyl Mercury in Mycelia after 4 Weeks of Growth

Hg^{2+} mM	0.06	DL-Homocysteine 0.32	0.64	3.2	mM
0.05	58	11	7	11	
	33.4	4.9	3.9	5.6	
0.10	5	29	24	12	
	3.9	18.0	11.3	5.7	
0.20	—	13	380	132	
		8.7	232	67	
0.40	—	—	37	710	
			19.3	214	
0.60	—	—	—	455	
				121	
1.0	—	—	—	345	
				101	

Concentration (upper figure) is given in ng Hg/g cells and total amount (lower figure) in ng of methyl mercury in mycelia after 4 weeks of growth. The average of two replicates is given in each case; —, no growth.

was added together with approximately equimolar amounts of DL-homocysteine and Hg^{2+} , the yield of methyl mercury was doubled. No similar increases in yield were noted either when DL-homocysteine was present in large surplus over Hg^{2+} (other factors being unchanged), or when DL-homocysteine was replaced by L-cysteine.

From these results we have drawn the following conclusions. First, the detoxicating methylation of mercury involves one or more steps of the methionine biosynthesis pathway¹⁰. Second, the finding that, out of five different thiols tested, only cysteine and homocysteine increase the amount of methyl mercury per cell weight, rules out the explanation that thiols stimulate methylation of mercury only by facilitating the uptake of Hg^{2+} to the cells. Third, the relationship between yield of methyl mercury and concentration of homocysteine and homoserine in the medium indicates that a negative control of the methylating enzyme—for example, a transmethylese¹⁰—is affected presumably by methionine (compare ref. 11). The methionine can probably be formed in sufficient amounts only when a large surplus of homocysteine (over Hg^{2+}) is available. On the basis of these conclusions we propose a tentative model for one type of biological methylation of mercury.

The methyl group, whether synthesized *de novo* or not⁴, is transferred to the mercury atom which is complexed to homocysteine. The methylation of mercury might then be regarded as an "incorrect" synthesis of methionine. As the methylmercury-homocysteine complex presumably cannot execute the feed-back control of the methylating enzyme(s), the synthesis continues until unloaded homocysteine molecules in sufficient amount have been methylated to methionine. Methionine seems to inactivate the enzyme or to repress the synthesis of it. This is observed when methionine is added to the medium, leading to a break in the detoxification of mercury by methylation. In this connexion it is quite plausible that the mutants resistant to Hg^{2+} are constitutive mutants. The control of one of the last enzymes in the methionine biosynthesis would then be impaired, giving rise to a continuous methylation of Hg^{2+} .

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Heterotransplantation of Cultured Cell Lines in Newborn Hamsters treated with Antilymphocyte Serum

CELL lines derived from neoplastic tissue differ from those from normal adult tissue¹ in their capacity to survive and grow in the cheek pouch of a hamster treated with cortisone, and this difference is now the basis of a test for potential malignancy. We have found a simple and rapid alternative in the use of hamsters treated with antilymphocyte serum (ALS) and this may be valuable in the screening for tumorigenicity in human diploid cell strains used in the production of virus vaccines^{2,3}. ALS is an effective suppressor of immune responses to skin allografts⁴, of tumour induction by oncogenic viruses⁵ and of the development of metastases of allogeneic tumour grafts⁶. It is also known that tumours are formed in ALS-treated weanling mice after subcutaneous inoculation of cultured human tumour cells^{7,8}. It was this that suggested that ALS may provide an improved alternative to standard methods of host conditioning for tumour transplantation.

Antilymphocyte serum was prepared by injecting New Zealand white rabbits subcutaneously with 10^9 viable, adult hamster thymocytes in Freund's complete adjuvant on day 0, followed by two intravenous injections of $3-5 \times 10^8$ thymocytes on days 14 and 28. Rabbits were bled 7 days after the last injection. Sera were subjected to a temperature of 56°C for 30 min and tested for haemolytic and cytotoxic antibody. Haemolytic antibody titres were determined by incubating 0.1 ml. of each two-fold dilution of ALS with 5×10^6 hamster erythrocytes and 0.1 ml. of guinea-pig complement. Since none of the ALS prepared had significant haemolytic activity (titre $<1:10$), it was used unabsorbed for injection into newborn hamsters. Cytotoxic antibody tests were performed by incubating 0.1 ml. of each dilution of ALS with 0.8 ml. of NCTC 109 (ref. 9) medium containing 5×10^6 hamster thymocytes and 0.1 ml. of guinea-pig complement. After incubation at 37°C for 45 min, living and dead cells were counted by a haemocytometer, using 0.01% eosin; the endpoint was expressed as the dilution which killed approximately 50% of the cells. The cytotoxic antibody titre of the ALS used in these experiments was 1:640.

Human epidermoid carcinoma (KB) cells¹⁰ (from Dr George Foley) were collected from culture flasks with 0.05% trypsin and suspended in Eagle's minimal essential medium¹¹ at concentrations ranging from 5×10^4 to 5×10^5 cells per 0.1 ml. In the same way, cells were prepared from primary cultures of rhesus monkey kidney and hamster embryo, from cultures of human foetal diploid fibroblasts (strain WI-38, ref. 12), and from two heteroploid cell lines, *Cercopithecus* monkey kidney BS-C-1 (ref. 13) and rhesus monkey kidney LLCMK₂ (ref. 14).

Syrian hamsters (*Mesocricetus auratus*) approximately 24 h old were inoculated subcutaneously with cell suspensions (0.1 ml.) just above the tail towards the back and neck, and immediately after inoculation, 0.95 ml. of ALS or normal

rabbit serum (NRS) was injected intraperitoneally. All subsequent injections of ALS or NRS—0–4 additional injections, 3–7 days apart—were given subcutaneously in 0.1 ml. doses. Cell suspensions in 0.1 ml. were also inoculated intradermally into the cheek pouches of adult, 60–70 g Syrian hamsters by the technique of Foley and Handler¹. Adult hamsters were injected subcutaneously with 2.5 mg of cortisone in 0.05 ml. once on the day the cells were inoculated and then twice a week. All hamsters were observed once each week for at least 4 weeks. Three diameters of the tumours were measured with a graduated caliper and the tumour volume was expressed as 0.52 of the product of the diameters in mm^3 (ref. 7). Selected animals in each experiment were killed and their tissues were preserved for histological examination.

Table 1 Growth of KB Cells in Newborn Hamsters treated with ALS or NRS

KB cell inoculum	Total injections of ALS or NRS	NRS treatment		ALS treatment	
		No. with tumour		No. with tumour	
		No. inoculated	Week 3	No. inoculated	Week 3
5×10^5	5	0/6	1/6 †	9/9	9/9 *
5×10^5	3	1/9	2/9 †	7/7	7/7 *
2.5×10^5	2	2/9	1/9 §	8/12	10/12 *
2.5×10^5	1	—	—	11/14	11/14 *
5×10^4	5	0/7	0/7	0/4	3/4 †
5×10^4	3	0/8	0/8	3/4	4/4 †

* Large metastatic tumours.

† Large tumours without metastases.

‡ Small tumours.

§ Small regressing tumours.

The first group of five injections (top of second column) was administered on days 0, 3, 6, 10 and 13; the second group of three was administered on days 0, 3 and 10; the third group of two was administered on days 0 and 7 and the fourth was administered on day 0.

The results (Table 1) indicate that a single injection of ALS is sufficiently immunosuppressive to permit progressive growth of large metastatic tumours in eleven out of fourteen newborn hamsters within 2 weeks of receiving 2.5×10^5 KB cells. Tumours in this group of animals grew to sizes ranging from 260–12,168 mm^3 by the third week. Forty-four out of fifty newborn hamsters treated with ALS and KB cells developed large, progressively growing tumours. Two of these died after 3 weeks and most survivors were runted and moribund. Nine had a subcutaneous growth of carcinoma with many mitotic figures and four had metastases to the lung and possibly the liver. Five of the thirty-nine control newborn hamsters treated with KB cells and NRS developed small tumours (260–520 mm^3) but they remained healthy and normal in size, and one tumour had regressed by the third week. Cortisone-treated adult hamsters receiving 2.5×10^5 KB cells also developed progressively growing tumours 50–2,106 mm^3 in volume by the third week. Two of twelve untreated adult hamsters receiving KB cells developed small regressing nodules 0.5–3.0 mm^3 in volume.

On the basis of these results, we chose an inoculum of 2.5×10^5 KB cells for both newborn and cortisone-treated hamsters and a single injection of ALS as the standard positive control in experiments designed to assess the tumorigenic potential of selected cell cultures in newborn hamsters. Cells tested for tumorigenicity were collected from cultures of primary hamster embryo, primary rhesus monkey kidney, human foetal lung strain WI-38, *Cercopithecus* monkey kidney line BS-C-1 and rhesus monkey kidney line LLCMK₂. In a series of tests, none of these were tumorigenic when tested at inocula of 10^6 cells in ALS-treated newborn hamsters, whereas 2.5×10^5 KB cells have consistently produced large, progressively growing tumours when tested simultaneously. Newborn hamsters treated with ALS and receiving cell inocula other than KB have remained healthy and normal in size,

demonstrating the lack of toxicity of ALS for the suckling hamster in these experiments.

The ALS used, although produced in rabbits with adjuvant, had an insignificant amount of red cell haemolysins and was non-toxic when administered unabsorbed to the suckling hamster. The induction of susceptibility to the growth of human KB carcinoma cells has also provided a simple means of assaying potency of ALS used in the hamster. Because other tests for cytotoxicity—for example, leucocyte agglutination and lymphoblast transformation and *in vivo* lymphocyte depletion—fail to measure the immunosuppressive activity of ALS accurately¹⁵, this new assay may be useful in the preparation and purification of effective ALS.

The failure of cultured cells to produce tumours in the immunosuppressed hamster does not preclude the possibility that such cells have undergone morphological or chromosomal changes (or both) during cultivation *in vitro*¹⁶. On the other hand, a marmoset diploid cell strain has been shown to be tumorigenic in an autologous host¹⁷. It would seem, therefore, that cytogenetic analyses, morphological studies and *in vivo* assays for tumorigenicity should be viewed as complementary tests, for any one alone produces insufficient information for critical evaluation of the malignant potential of a given cell strain.

The newborn hamster treated with ALS seems to be more sensitive than the cortisone-treated adult for the purposes of testing heterotransplantability of cultured cells because the tumours produced by the same inoculum of KB cells were significantly larger in animals treated with ALS. The procedure is also simple: very small doses of ALS are required and one injection suffices. Subcutaneous cell inocula are easily administered to the unanaesthetized animal and tumour formation can be assessed rapidly by visual observation of the caged hamster litters.

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Evidence for the Difference between the Odours of the Optical Isomers (+)- and (−)-Carvone

THE difference between the odours of the two optical isomers of carvone has been recognized for some time; (+)-carvone (isolated from caraway oil) was characterized as caraway-like while (−)-carvone (isolated from spearmint oil), as spearmint-like¹. Samples purified by gas-liquid chromatography with very high chemical and optical purity still exhibited the two characteristic odours (refs. 1 and 2 and L. Friedman, private communication). Moreover, (−)-carvone was synthesized from (+)-limonene and was found to have a spearmint odour^{1,2}. The possibility that a minor impurity of very low threshold and low concentration is responsible for the observed dissimilar odours has not, however, been completely eliminated.

Both the (+)- and (−)-carvones were synthesized unequivocally with high chemical and optical purity. (+)- and (−)-limonenes (Fluka) were carefully distilled to a purity >99.9% (by gas-liquid chromatography) and exhibited rotations of $[\alpha]_D^{27} +116.4$ and -106.4° (about 0.77 and 0.84, respectively, 95% ethanol) ($[\alpha]_D^{20} +126.8^\circ$ and -122.6° (neat), respectively³). Following the procedure of Royals and Horne⁴, the (+)- and (−)-limonenes were converted to (−)- and (+)-carvone, respectively. An investigation of the odours of (+)- and (−)-limonenes is under way. The natural and synthetic samples of the two optical isomers ((+)-carvone from Fritzsche Brothers; (−)-carvone from Chemical Procurement Laboratories) were prepared for sensory studies by distillation and preparative gas-liquid chromatography to a purity of >99.9% (for rotations see Table 1). All four samples had identical spectroscopic properties (infrared, nmr, m/e and ultraviolet).

The odour characteristics of the natural and synthetic carvones were evaluated by an odour panel of twenty-one to twenty-six persons who had extensive experience and proven reliability in odour judgments. All judgments were conducted in a room equipped with individual booths and supplied with odour-free air at constant temperature and relative humidity (RH) ($22^\circ \pm 1^\circ$ C and 50% RH).

Odour detection thresholds of the pure compounds were determined by procedures described before⁶. The natural and synthetic (−)-carvones had identical thresholds (2 parts of compound per 10⁹ parts of water) while the (+)-carvone from caraway had a somewhat lower threshold (85) than the synthetic (+)-carvone (130). This may be due to a very slight impurity from the natural source, but in any event the (−)-carvones are substantially stronger odorants than the (+)-carvones on a threshold basis. This in itself indicates a fundamental difference in the odour properties of the (+)- and (−)-carvones. These threshold values and simple odour ranking tests were used to equalize odour intensity of the isomers for the odour character tests. For these tests the concentration of (−)-carvone was 1 p.p.m. v/v and (+)-carvone 5 p.p.m. v/v.

Table 1 shows that a highly significant proportion of the judgments ($P \leq 0.01$)⁷ identified the (−)-carvone as more like the spearmint oil standard and (+)-carvone as more like the caraway oil standard. Furthermore, the (+)- and (−)-carvones from natural and synthetic sources gave essentially the same results, indicating that the characteristic odour is indeed due to the difference between the configurations of the (+)- and (−)-carvone molecules rather than to impurities from natural sources. In support of this evidence, no significant differences were shown between natural and synthetic sources of the isomers. When the judges were presented with single samples containing (+)- or (−)-carvone from natural or synthetic sources, they were able to identify the (+)-carvone and the (−)-carvone as having characteristic caraway and spearmint oil odour, respectively. Direct triangular comparisons between the odours of (+)- and (−)-carvones from natural and synthetic sources showed no significant differences as a

Table 1 Comparison of (+)- and (-)-Carvones with Standard Samples of Spearmint and Caraway Oil

Comparison *	[α] _D ²⁷ † (deg)	lit. [α] _D ²⁷ ‡ (deg)	Standard	N	No. who identified sample as most like standard	
					(-)-carvone	(+)-carvone
(-)-Carvone (L) against (+)-carvone (L)	-59.2 +58.3	-62.46 +62.32	Spearmint	25	21	4
(-)-Carvone (S) against (+)-carvone (C)	-54.2 +59.6		Spearmint	22	17§	5
(-)-Carvone (S) against (+)-carvone (C)			Caraway	25	5	20§
(-)-Carvone (S) against (+)-carvone (L)			Caraway	22	5	17§

* (L) Synthesized from limonene, (S) purified from spearmint oil, (C) purified from caraway oil.

† Concentration 0.22, 0.24, 0.45, 0.26, respectively, 95% ethanol.

‡ Neat⁵.

§ $P \leq 0.01^7$.

|| $P \geq 0.001^7$.

result of the source (natural or synthetic) of either isomer but very highly significant differences ($P \leq 0.0001$)⁷ between (+)- and (-)-carvones from both sources.

Thus, the experimental evidence is in favour of the claim that the optical isomers of carvone have characteristically different odours; the (-)-carvone resembling spearmint oil and the (+)-carvone, caraway oil.

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Crystallization of a Second Adenovirus Protein (the Fibre)

THE adenovirus capsid has icosahedral symmetry, and is constructed from 252 capsomeres¹. In all, 240 hexons define the three-fold and two-fold positions on the faces and edges of the icosahedron, and there are twelve pentons (each consisting of a penton base and a projecting fibre) at the five-fold vertices^{2,3}. Cells infected with adenovirus produce free hexons, pentons and fibres as well as intact virions, and these free "soluble antigens" have been purified by several methods (see review by Schlesinger⁴). Crystallization of the hexon has already been described⁵ and we report here a procedure for purification and crystallization of the fibre.

Human adenovirus type 5 (Ad 5) was propagated in monolayers of KB cells, and the soluble antigens were extracted as previously described⁶. Briefly, fluoro-carbon extracts of infected cells were centrifuged in caesium chloride density gradients to band the virus, and the material remaining above the virus band provided the source of soluble antigen. The soluble antigen fraction collected from sixty 20 ounce bottles of infected cells contained approximately 200 mg of protein. After dialysis against 0.01 M phosphate buffer, pH 6.8, this fraction was applied to a 3 × 12 cm column of DEAE 'Sephadex A-50' (Pharmacia), equilibrated in the same buffer. The bound proteins were eluted stepwise with increasing concentrations of sodium chloride; the fractions were assayed for protein by the Lowry method and the antigenic activities were identified by micro-immunodiffusion against antiserum to whole virus⁷. The fibre and penton fractions were pooled and concentrated by overnight vacuum dialysis against 0.01 M phosphate buffer pH 6.8 to a final volume of approximately 5 ml.

Tryptic digestion of the penton results in breakdown of the penton base, while the fibre remains intact⁸, and this procedure was used to increase the supply of fibre, albeit at the expense of intact penton. The fibre plus penton concentrate was incubated with trypsin (1 part enzyme : 10 parts substrate by weight) for 30 min at 37° C in 0.1 M NaCl, 0.01 M borate buffer, pH 9.0. After 24 h dialysis against 0.01 M phosphate buffer, pH 6.8, at 4° C, the residual fibre was rechromatographed, the peak fractions were pooled and reduced to a final volume of 1 ml. by vacuum dialysis against the same buffer. When the concentrate was then dialysed against 0.01 M phosphate buffer, pH 6.0, the fibre aggregated, and streaming birefringence was visible to the naked eye. Examination in the light microscope revealed needle shaped crystallites, up to 50μ long (Fig. 1).

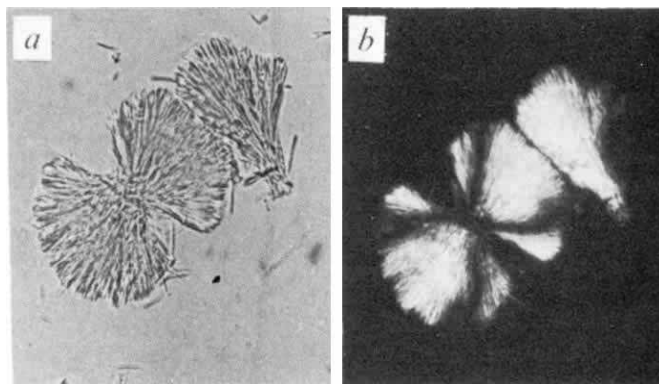


Fig. 1 *a*, Photomicrograph of aggregates of small crystals ($\times 240$); *b*, the same field viewed in the polarizing microscope with crossed polaroids: note the cross of extinction demonstrating the birefringent nature of the material.

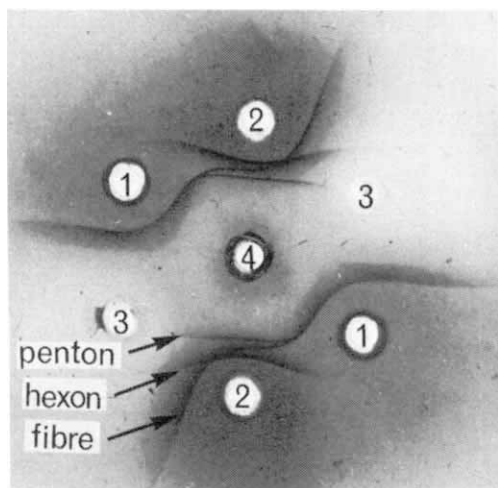


Fig. 2 Micro-immunodiffusion. 1, Antiserum to crystals of Ad 5 fibre; 2, antiserum to Ad 5 virus; 3, Ad 5 fibre, redissolved crystals; 4, Ad 5 soluble antigens.

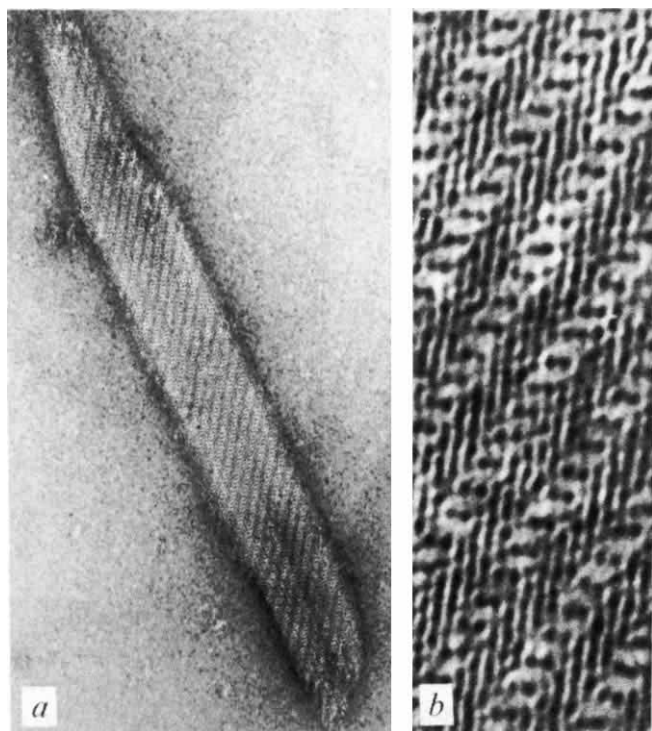


Fig. 3 *a*, Electron micrograph of Ad 5 fibre single crystallite, negatively stained with uranyl acetate ($\times 75,000$); *b*, detail of the same crystallite at higher magnification ($\times 500,000$).

Various observations have confirmed that the crystals are composed only of fibre units. The crystals sediment rapidly during low speed centrifugation (2,000 r.p.m. in a bench centrifuge) and only a small proportion of the protein remains in the supernatant. After washing twice with the same buffer and then with distilled water, the crystals readily redissolve in 0.1 M NaCl, 0.01 M borate buffer, pH 9.0. Polyacrylamide electrophoresis in the presence of sodium dodecyl sulphate⁹ shows that the washed crystals contain a single species of polypeptide chain with a molecular weight in the range 60–65,000, and the mother liquor shows traces of two minor bands corresponding to degraded penton base, as well as fibre¹⁰. The washed crystals give a single precipitin line in micro-immunodiffusion against adenovirus type 5 antiserum, and antiserum raised in rabbits against crystallized fibre also shows identity with this line (Fig. 2).

Although the crystals are too small to provide useful X-ray diffraction patterns, they may be visualized in the electron

microscope, using uranyl acetate as negative stain (Fig. 3*a*). Fine structure down to 3.5 nm may be discerned in high resolution micrographs (Fig. 3*b*) and preliminary optical diffraction studies indicate a highly ordered structure that will be amenable to detailed analysis.

Crystallization provides a useful method for preparation of the adenovirus type 5 fibre and should enable further work on the structure and function of this protein to proceed from an established criterion of purity.

We thank Mr M. R. Young for the photomicrograph, Dr E. J. Wills and Miss P. M. Bennett for the electron micrographs.

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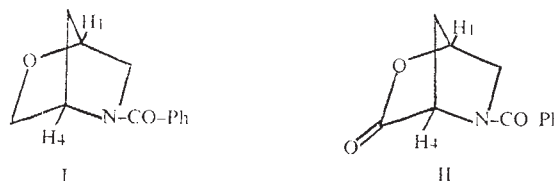
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Interaction between an Amide Nitrogen Lone Pair and a Noncontiguous Carbonyl Carbon and its Effect on Amide Rotation

ALTHOUGH rotational barriers^{1–4} about the C–N bond in simple amides have been investigated extensively by means of nuclear magnetic resonance (NMR) spectroscopy, there is no information about the effect of proximal dipolar groups on the rotational process. Information of this type would be particularly relevant to peptide bonds in proteins because of the multiplicity of dipoles with the potential for Coulombic interaction with the amide moiety, and because such interaction may be important in determining the preferred conformation of proteins⁵. We have evidence to suggest that N–CO rotation is significantly increased by orbital overlap between the nitrogen atom of the amide group and a noncontiguous carbonyl function.



The compounds, I and II, that were studied are derivatives of 2-oxa-5-aza-bicyclo[2.2.1]heptane and were prepared as follows. Hydroxy-L-proline was converted to III in Schotten-Baumann conditions and then esterified with diazomethane. Tosylation of IV in pyridine gave V which was reduced to alcohol VI with LiBH_4 . Cyclization to I, melting point $76^\circ\text{--}78^\circ\text{C}$, $[\alpha]_D^{25} + 27.8^\circ$ (c. 1.56, EtOH) was achieved by refluxing VI in MeOHMe-ONa for 2 h. The calculated analysis for $\text{C}_{12}\text{H}_{13}\text{NO}_2$ was C, 70.91; H, 6.45; N, 6.89; we found

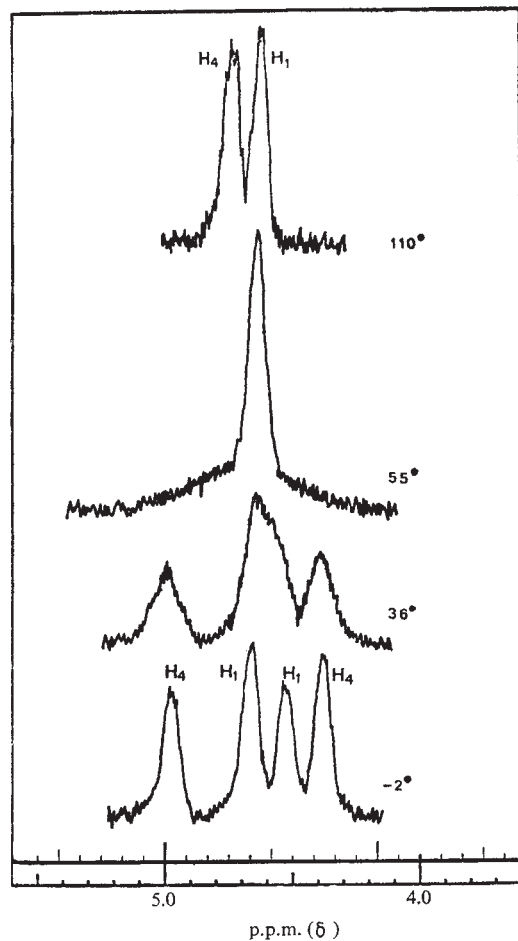
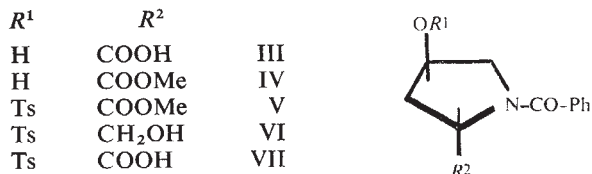


Fig. 1 Bridgehead proton resonances for N-benzoyl-2-oxa-5-azabicyclo-[2.2.1]heptane (I) in CDCl_3 .

C, 71.21; H, 6.57; N, 6.74. Saponification of V with NaOH in dioxane at 4° C produced the acid VII, which was converted to lactone II, melting point 132°–134° C, $[\alpha]_D^{20} + 91.2$ (c. 1.25, EtOH), according to the method of Patchett and Witkop⁶. The calculated analysis for $\text{C}_{12}\text{H}_{11}\text{NO}_3$ was C, 66.35; H, 5.15; N, 6.40; we found C, 66.61; H, 5.25; N, 6.35.



The -2° NMR spectrum of (I) shows two sets of signals corresponding to H_1 and H_4 (Fig. 1). The peaks in each set have approximately equal areas. The coalescence temperatures for these signals are 35.5° and 55° C, respectively. The

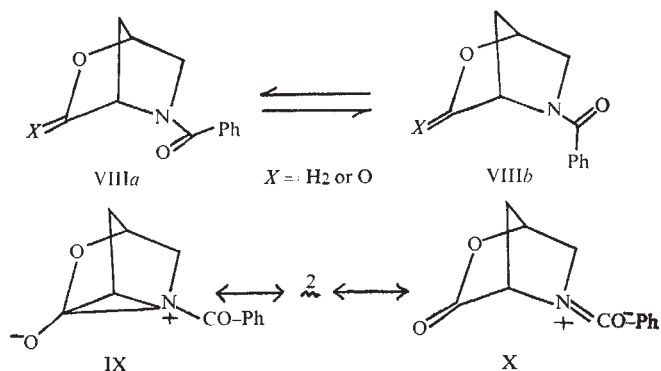


Table 1 NMR and Activation Data for Conformational Interconversion of I and II

Compound	Bridge-head protons	$\Delta\nu$ (Hz) *	T_c †	k (s^{-1})	$\Delta G^\ddagger$ (kcalories/mol) ‡
I	H_1	8§	35.5°	18	16.3 ± 0.2
	H_4	36§	55°**	80	16.9 ± 0.5
	H_1	10¶	-4° ††	22	14.1 ± 0.5
II	H_4	29¶	12° ††	64	14.2 ± 0.3

NMR data were determined at 60 MHz in CDCl_3 .

* Maximum peak separation.

† Coalescence temperature ($^\circ\text{C}$).

‡ Values are the mean of 5 determinations.

§ Obtained at -2°C .

¶ Obtained at -30°C .

|| Obtained from direct observations of coalescence; thought to be correct within $\pm 2^\circ\text{C}$.

** Obtained from maximum peak width at half-height; due to the broadness of this peak and interference by the H_1 signal; T_c is thought to be correct within $\pm 3^\circ\text{C}$.

†† Obtained from maximum peak width at half-height; thought to be correct within $\pm 2^\circ\text{C}$.

-30° spectrum of the lactone II also has four peaks for these protons (Fig. 2). Each set of two peaks possesses an area ratio of approximately 0.3 : 1 with coalescence temperatures at -4° (H_1) and 12° C (H_4).

The mean lifetimes ($2T$) for rotamers VIIIa and VIIIb were calculated according to the method of Gutowsky and Holm³. The Eyring formulation was used to calculate values of $\Delta G^\ddagger$ for the rotation process⁷. The $\Delta G^\ddagger$ values (Table 1) disclose that conformational interconversion, VIIIa $\rightleftharpoons$ VIIIb, occurs with greater facility in the case of lactone II. Although this can be due to differences in $\Delta S^\ddagger$ and/or $\Delta H^\ddagger$, it is more probable

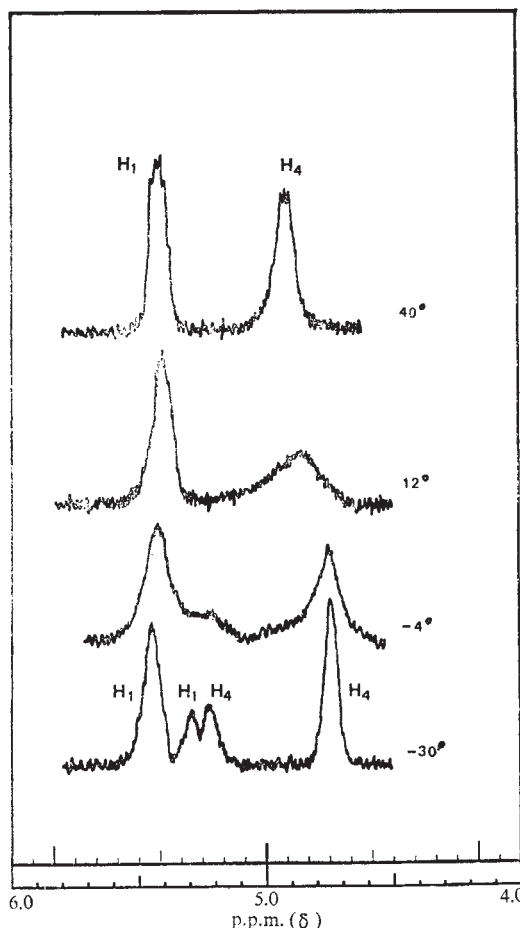


Fig. 2 Bridgehead proton resonances for N-benzoyl-2-oxa-5-azabicyclo-[2.2.1]heptane-3-one (II) in CDCl_3 .

that the observed difference, $\delta\Delta G^\ddagger \sim 2$ kcalories/mol, arises primarily from the latter, for I and II have a rigid ring system.

We propose that the nitrogen lone pair enters into a transannular interaction⁸ with the lactone carbonyl to form a resonance contributor IX which is analogous to the delocalized resonance form of homoenolate ions proposed by Nickon *et al.*⁹. This would tend to reduce X as a resonance contributor and thereby facilitate internal rotation as a result of decreased Sp^2 character of the N-C bond. The higher amide carbonyl stretching vibration ($1,625\text{ cm}^{-1}$) for II, when compared with I ($1,613\text{ cm}^{-1}$) (CHCl_3 solvent), is consistent with this interpretation.

The fact that the bicyclic ether possesses approximately equal populations of conformers VIIIa and VIIIb ($X=\text{H}_2$) while the lactone ($X=\text{O}$) rotamers are unequally populated suggests that dipole-dipole interaction plays a significant part in determining the orientation of the amide carbonyl group. It is likely that VIIIa ($X=\text{O}$) is favoured, for the carbonyl dipoles in that conformation should cancel one another. Significantly, the CO-C-N-CO- geometry of VIIIb is similar to the conformation¹⁰ usually found in proteins. Coulombic interaction similar to IX should be of smaller magnitude in polypeptides because of greater intergroup distance when compared with those in the bicyclic system. But because the decay of Coulombic attraction with increasing distance is relatively small it may conceivably play a significant part in determining protein conformation. Moreover, if polypeptide folding brings distant dipoles into close proximity, it is possible that the "stiff" peptide linkage involved in the interaction may become more freely rotating.

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Recommended Procedures for testing Genetic Hazards from Chemicals, based on the Induction of Dominant Lethal Mutations in Mammals

GENETIC hazards from drugs and chemical pollutants are now widely recognized and appropriate, sensitive and economic mammalian methods for detecting and measuring mutagenic effects of chemicals have been developed. These methods are relevant to man¹ and include *in vivo* cytogenetics, host-mediated assay, and dominant lethal assay; sub-mammalian systems are generally considered to be of ancillary value only. The Advisory Panel on Mutagenicity of Pesticides¹ and the Food and Drug Administration Advisory Committee on Protocols

for Safety Evaluation² have recommended that pesticides and food additives should be tested for mutagenicity in mammalian systems before registration. This communication recommends practical procedures for the dominant lethal assay, and indicates how these can be integrated in routine toxicological practice.

Dominant lethal mutations indicate major genetic damage and have been used in mammals for measuring the effects of X-rays and chemical mutagens. Data from these systems can be extrapolated to man, especially as many recognizable human autosomal traits are due to dominant lethal mutations. The genetic basis for dominant lethality is chiefly the induction of structural and numerical chromosomal aberrations, such as translocations and aneuploidies, which may sequentially induce pre-implantation losses of non-viable zygotes, early foetal deaths, and sterility and semi-sterility in F_1 progeny³⁻⁵. Such chromosomal aberrations have been identified in F_1 progeny of male rodents treated with mutagens⁶⁻¹⁷, and also in human abortions and congenital malformations¹⁸.

In the dominant lethal assay, male mice or rats are dosed singly with sub-toxic concentrations of the drugs to be tested. They are then mated sequentially with groups of untreated females. Matings in weeks 1-2, 3-4 and 5-8 after treatment of male mice respectively represent samples of post-meiotic, meiotic, and pre-meiotic stages of spermatogenesis; corresponding periods in the rat are 1-4, 5-6 and 7-10 weeks. Timing of stage sensitivity can be complicated by delayed metabolic activation or detoxification of the drug, or by drug-induced inhibition of mitosis or meiosis. Females are inspected daily for vaginal plugs, dissected on approximately the fourteenth day of pregnancy and scored for corpora lutea and for total implants comprising early and late foetal deaths and living foetuses. Mutagenic effects are expressed conventionally as the mutagenic index (early foetal deaths/total implants) $\times 100$. The dominant lethal assay has recently been modified and simplified for large scale routine testing in mice¹⁹. Inspection for vaginal plugs is omitted and, instead, all females are dissected 13 days after the mid-week of their caging and presumptive mating. Corpora lutea counts, which are difficult and relatively imprecise in mice, are also omitted and pre-implantation losses are scored by contrasting values of total implants in females mated with treated and control males.

Detailed characterization of the reproductive parameters of large control populations is essential to the development of standard protocols for the dominant lethal assay. Such studies will define the range and cyclic variation in total implants, pre-implantation losses and early and late foetal deaths. Closed colony, random-bred rodents are suitable for these assays and the use of F_1 hybrids may minimize variation. Assays should be conducted on both mice and rats as standard toxicological practice. Species variation in sensitivity has already been noted; for example, DDT does not induce either pre-implantation losses or early foetal deaths in mice (S. S. Epstein, unpublished data) but does so in rats (M. Legator, unpublished data). Chemicals should be tested for mutagenicity after acute, sub-acute and chronic administration to male rodents by oral, parenteral and respiratory routes (Table 1).

Table 1 Recommended Scheme for Dominant Lethal Testing in Male Mice

1. Acute
Single oral or parenteral administration at $1/5$ th of LD_{50} s to male mice mated with three females each for 5 successive weeks, with undosed controls.
2. Sub-acute
Oral or parenteral administration at $1/5$ th of LD_{50} s for 5 successive days to male mice mated with three females each for 5 successive weeks, with matching controls.
3. Chronic
Oral administration at maximally tolerated doses for 3 months minimally, to male mice mated with three females each for 4 successive weeks, with matching controls.

The route of test should reflect human exposure. Sub-acute testing is recommended largely to anticipate and reflect the role of possible hepatic microsomal detoxification or activation, while the object of chronic administration is to detect spermatogonial mutations. Enough animals must be used so that a test which indicates differences significant at the 5% level will have a 99% probability of detecting any true difference that exceeds 20% of the control mean number of living implants. For initial testing, a single dose is adequate. In acute and sub-acute testing 0.2 of the LD₅₀s are recommended while maximally tolerated doses are appropriate for chronic testing.

Use of the modified assay may be recommended for screening in mice. In rats, however, matings should be timed and corpora lutea counted. Complete autopsy of females is essential as intercurrent infection in any animal can induce pre-implantation losses and early foetal deaths²⁰. The induction of dominant lethal mutations is scored directly by an increased incidence of early foetal deaths and indirectly by an increased incidence of pre-implantation losses measured by the difference between total implants in control and test females, and/or by the difference between corpora lutea counts and number of total implants. Mutagenic effects may be reported directly in terms of the mutagenic index and/or as the number of early foetal deaths per pregnant mouse, or indirectly in terms of pre-implantation losses. As numerator and denominator are contributory to the mutagenic index, estimates of standard deviation are complex. Initial testing may reasonably be restricted to meiotic and post-meiotic stages, for no chemical has been shown to induce dominant lethal mutations exclusively in pre-meiotic stages. While various chemicals induce pre-meiotic effects, as measured by early foetal deaths and/or pre-implantation losses³⁻⁵, these also produce marked meiotic and/or post-meiotic effects. Mutagenic effects in acute, sub-acute, or chronic testing, as measured directly by increased early foetal deaths and/or indirectly by pre-implantation losses, should be confirmed by subsequent testing *inter alia* over extended dose ranges. The dominant lethal assay can also be used to investigate the possibility of synergistic, antagonistic, or other interactive mutagenic effects; for example, caffeine is non-mutagenic and does not synergize the mutagenic effects of X-rays or chemical mutagens in mice²¹.

In vivo mammalian tests for mutagenicity could be practically and economically integrated in routine toxicity testing. In acute, sub-acute and chronic toxicity testing, for example, or in the course of carcinogenicity tests, male mice or rats may be mated with untreated females which can subsequently be examined for the induction of dominant lethal mutations, indicated by early foetal deaths and pre-implantation losses. Cytogenetic analyses in rats can be performed serially on peripheral blood, on bone marrow and possibly on testes at death as a standard procedure in toxicity testing. An interesting suggestion in this context is that testing for toxicity, teratogenicity, carcinogenicity and mutagenicity could be performed on the same groups of animals²². Finally, in common with all toxicological procedures, the protocols recommended here should reflect dynamically technical and conceptual advances.

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Carcinogenicity of Industrial Chemicals Propylene Imine and Propane Sultone

PROPYLENE imine is an important chemical intermediate with a variety of applications in the production of polymers, coatings, adhesives, textiles and paper finishes. Likewise, propane sultone has many potential uses in the detergent, textile, rubber, pharmaceutical and agricultural chemical industries. Their chemical reactivity suggests that these compounds may have deleterious effects in living systems. Ethylene imine, closely related to the higher homologue, propylene imine, is known to have carcinogenic, mutagenic and teratogenic activity¹⁻³. A preliminary report dealing with the carcinogenicity of propane sultone has appeared⁴.

Range-finding experiments have shown that the maximal tolerated doses of propylene imine and propane sultone, in distilled water, administered by gavage twice a week to 6 week old male and female Charles River CD rats, were 20 mg/kg and 56 mg/kg, respectively. Chronic tests were also carried out at these dose levels, and at 50% of these levels, that is, 10 mg/kg for propylene imine, and 28 mg/kg for propane sultone, with groups of twenty-six male and twenty-six female rats at each level. There were similar control groups. Rats given propylene imine (20 mg/kg) suffered from advanced flaccid paralysis after 18 weeks and the mortality rate was high. At the lower dose, paralysis occurred at a lesser extent after 30 weeks. Because of the general condition of the animals and the appearance of palpable masses, administration of propylene imine at the high dose was discontinued after 28 weeks. The pattern of survival of the rats given propane sultone was similar. Here too, the 56 mg/kg dose was discontinued after 32 weeks. All surviving animals were killed and necropsied after 60 weeks.

Twenty-two of fifty-two male and female rats given 20 mg/kg of propylene imine had twenty-eight tumours, and thirty-seven of fifty-two rats given 10 mg/kg had forty-five tumours (Table 1). Brain tumours (gliomas) and squamous cell carcinomas of the

Table 1 Principal Types of Tumours induced in 60 Weeks by Twice Weekly Oral Administration of Propylene Imine and Propane Sultone

Compound Dose (mg/kg) Sex	Number of rats in groups of 26 with tumour							
	Propylene imine				Propane sultone			
	20*		10		56†		28	
	M	F	M	F	M	F	M	F
Breast	0	10	0	20	1	13	1	7
Glioma	3	1	4	2	16	13	12	15
Ear duct	3	0	3	3	3	3	1	0
Leukaemia	6	0	4	0	4	3	0	2
Intestine	2	0	2	0	3	1	4	1
Miscellaneous	0	3	4	3	4	6	5	7

* Dosing stopped after 28 weeks.

† Dosing stopped after 32 weeks.

ear duct were found in both sexes at both doses and disseminated granulocytic leukaemias were found in males. Many mammary adenocarcinomas were found in the female rats; more were observed in rats given the smaller dose, probably because this dose was continued for the entire experiment, whereas the larger dose was discontinued after a relatively short exposure. A number of the mammary tumours were multiple, some metastasizing to the lung. We conclude that propylene imine is a powerful carcinogen.

Propane sultone administered intragastrically at two dose levels gave rise chiefly to gliomas, with a similar incidence irrespective of dose and sex. In addition, there were several cases of leukaemias, ear duct tumours and adenocarcinomas of the small intestine, and one case of colon adenocarcinoma. In females there was a large number of mammary adenocarcinomas, related to the size of the dose, some of which metastasized to the lung. Because the sixty-four negative control animals also served as controls for concurrent studies, only some, six males and six females, were killed at 61 weeks. A pituitary chromophobe adenoma was discovered in one female control, but the remaining eleven were free from tumours. In addition, one female control died after 33 weeks of a cerebral glioma. A group of positive control animals treated with a known carcinogen responded in the anticipated manner.

Druckrey *et al.*⁴ emphasized that subcutaneous injection of propane sultone leads to local sarcomas in all treated rats. Their impression was that oral or intravenous administration was less effective, but nonetheless they also found tumours of the brain and nervous system. We agree that propane sultone is a potent carcinogen and that adequate precautions in handling the compound are necessary. Furthermore, we conclude that propylene imine is also a powerful carcinogen and that it affects a similarly wide range of organs, and that both substances are potent carcinogens when administered orally.

Note added in proof. Two papers dealing with the carcinogenicity of propane sultone have just appeared (Druckrey, H., *et al.*, *Z. Krebsforsch.*, **75**, 69; 1970; and Van Duren, B. L., *et al.*, *J. Nat. Cancer Inst.*, **46**, 143; 1971).

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Neutron Radiography of Osseous Tumours

THE use of a beam of neutrons to create an image of an object is a procedure with wide application¹⁻⁴. Neutron radiography provides a non-destructive diagnostic technique giving information not obtainable by density-dependent X-ray techniques. We believe we have developed the first effective neutron radiographic technique of detecting pathological tissue within unsectioned biopsied bone specimens.

The neutron source used in this study was a nuclear reactor with thermal neutrons appropriately collimated to form a beam with limited angular divergence ($\sim 1/100$ radians). After the neutron beam had penetrated the specimens, a 0.001 inch gadolinium screen was used to convert the neutrons to 70 keV electrons which created the photographic image on the X-ray film. Neutron radiographic images are produced by the interaction of a thermal neutron beam with the anatomic nuclei of the tissue cells of the specimen, whereas an X-radiograph is produced by the interaction of the electrons with the atoms of the specimen. Thus the contribution of neutron radiography is in the creation of images whose characteristics reflect the variation in hydrogen atom content, rather than simple density.



Fig. 1 An X-radiographic view of a resected pathological specimen of the ramus of a mandible showing a large radiolucent area involving a major portion of the anterior and middle region of the ramus. The exact nature and degree of extension into the surrounding bone of the radiolucent lesion are not discernible.

To demonstrate how neutron radiography can show an aberrant solid soft tissue mass within a bone cavity, a case of a large dentigerous cyst of the mandible containing a mural ameloblastoma is presented here. The radiographic views of the facial bones revealed bone destruction involving a portion of the ramus and body of the mandible, but not the degree to which this radiolucency represented benign cystic involvement, bone destruction in advance of tumour invasion or actual ameloblastic tumour invasion advancement along marrow vascular spaces (Fig. 1). Any surgeon would be faced with the decision of determining adequacy of bone margins which were not detectable by gross X-ray examination. The adequacy of the surgical margins as a rule is known only after decalcification of the bone surrounding the tumour mass in the surgical specimen. Neutron radiography in this case, how-

ever, revealed clearly that the tumour mass consisted of a distinct, thickly encapsulated wall with no invasion of the surrounding marrow vascular spaces of the bone (Fig. 2). A mass of tumour tissue was seen to be proliferating into the centre of the cyst, but did not seem to be invading normal bone surrounding the lesion. In this case, therefore, it might have been possible to remove the tumour by curettage. Histological examination of the decalcified surgical specimen corroborated the evidence of neutron radiography.

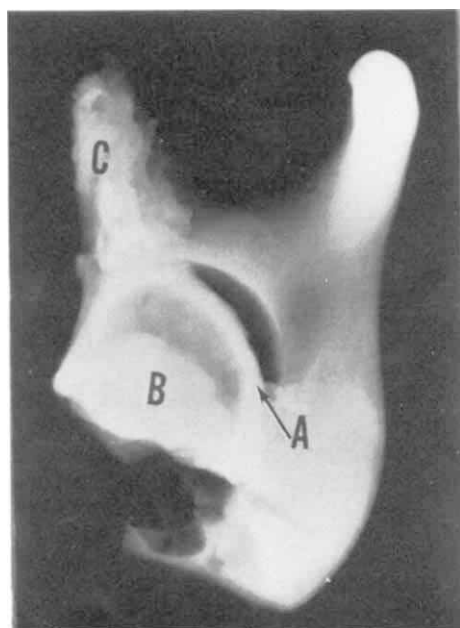


Fig. 2 A neutron radiographic view of the same specimen shown in Fig. 1. The contents of the radiolucent area are shown by the neutron radiographic view to be composed of: *A*, a thick encapsulating soft tissue wall surrounding a cyst; *B*, a solid tumour mass projecting from the cyst wall into the cyst lumen. Fragments of the temporalis muscle (*C*) can be seen still adhering to the coronoid process.

In other cases of tumours involving the facial bones, significant invasion of the tumour which had not produced sufficient bone destruction to be visible by routine X-ray views was revealed in the neutron radiographic images. Future development of neutron radiographic techniques may make possible *in vivo* imaging of tissue if the radiation dose from such neutron radiographic exposures can be reduced to permissible ranges.

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Effect of ACTH and Dibutyryl Cyclic AMP on Catecholamine Synthesizing Enzymes in the Adrenals of Hypophysectomized Rats

MAINTENANCE of normal levels of adrenal medullary enzymes concerned with the synthesis of catecholamines requires an intact pituitary-adrenocortical system. Wurtman and Axelrod^{1,2} showed that after hypophysectomy there is a striking decrease in adrenal levels of phenylethanolamine-N-methyl transferase (PNMT), the enzyme which converts noradrenaline to adrenaline. This decrease is prevented or reversed by treatment with ACTH or glucocorticoids. Dopamine- β -hydroxylase, which is responsible for the formation of noradrenaline from dopamine, was shown by Kvetňanský *et al.*³ to be decreased in the adrenal glands of hypophysectomized rats; levels of this enzyme are also restored by treatment with ACTH⁴ or dexamethasone (Gewirtz *et al.*, unpublished observations). Tyrosine hydroxylase, the enzyme which converts tyrosine to 3,4-dihydroxyphenylalanine (DOPA), is also diminished in the adrenals of hypophysectomized rats and restored by ACTH treatment^{3,5}. Unlike those of dopamine- β -hydroxylase and PNMT, however, tyrosine hydroxylase levels are not increased by treatment with dexamethasone⁵. Thus ACTH seems to have an effect on adrenal tyrosine hydroxylase which is not mediated by corticosteroids.

Table 1 Effects of ACTH and Dibutyryl Cyclic AMP on Adrenal Weight, PNMT Activity and Tyrosine Hydroxylase Activity in Hypophysectomized Rats

Group	Adrenal weight (mg/pair)	PNMT activity	Tyrosine hydroxylase activity
Sham-	38.9	41.70	66.91
hypophysectomized	± 0.72	± 1.14	± 4.02
Hypophysectomized	18.2	16.39	49.54
	$\pm 0.86^*$	$\pm 0.60^*$	$\pm 2.54^\dagger$
Hypophysectomized	37.0	37.19	64.76
+ ACTH	$\pm 1.35^\S$	$\pm 1.11^\ddagger$	$\pm 3.34^\S$
Hypophysectomized	21.8	19.25	79.31
+ dibutyryl cyclic AMP	$\pm 0.33^* \P$	$\pm 0.61^* \P$	$\pm 1.97^\ddagger \P$

Three days after hypophysectomy rats were begun on 5 days of treatment with ACTH (5 IU subcutaneously/day) or dibutyryl cyclic AMP (25 mg subcutaneously in 0.25 ml. 8% gelatin twice daily). Rats were killed 8 days after hypophysectomy. PNMT units are nmol metanephrine/pair of adrenals/h. Tyrosine hydroxylase units are nmol DOPA/h/pair of adrenals. Results are expressed as means $\pm$ s.e. for groups of six to eight rats.

* $P < 0.001$ compared with sham hypophysectomized control group.

$^\dagger P < 0.01$ compared with sham hypophysectomized control group.

$^\ddagger P < 0.02$ compared with sham hypophysectomized control group.

§ Not significantly different from sham hypophysectomized control group.

$^\P P < 0.001$ compared with hypophysectomized group.

$^\P P < 0.01$ compared with hypophysectomized group.

The effect of ACTH on steroidogenesis in adrenocortical cells seems to be mediated by cyclic AMP^{6,7} and both cyclic AMP and its dibutyryl analogue stimulate steroidogenesis in adrenal slices⁸. Furthermore, dibutyryl 3',5'-adenosine monophosphate (cyclic AMP) given to hypophysectomized animals increases the weight, DNA, RNA, and protein content of the adrenals and maintains adrenal cortical responsiveness to administered ACTH⁹. Since adrenal tyrosine hydroxylase levels in hypophysectomized rats are increased by ACTH but not dexamethasone, it is possible that cyclic AMP has a direct effect on tyrosine hydroxylase levels. The effects of dibutyryl cyclic AMP on the enzyme levels in the adrenals of hypophysectomized rats were therefore examined.

Hypophysectomized and sham hypophysectomized male rats weighing 220–230 g were obtained from the Hormone Assay Laboratories, Inc. (Chicago, Illinois). Three days after hypophysectomy treatment with N₆O₂-dibutyladenosine-3'-5'-cyclic phosphate monosodium · 5H₂O (Calbiochem, Los Angeles, California), 25 mg subcutaneously/rat in 0.25 ml. 8% gelatin every 12 h; ACTH (Acthar gel), 5 IU subcutaneously/rat/day; or dexamethasone sodium phosphate (Decadron phosphate injection), 1 mg subcutaneously/rat/day, was begun. After 5 days of treatment the rats were killed by cervical fracture and the adrenal glands rapidly removed, cleaned, weighed and homogenized in 1.0 ml. ice-cold isotonic sucrose. An aliquot (100 λ) of the homogenate was added to 200 λ of 0.15% 'Triton X-100'; 50 λ of this mixture was assayed for dopamine- β -hydroxylase by the method of Friedman and Kaufman¹⁰ as modified by Viveros *et al.*¹¹. The remainder of the homogenate was centrifuged at 26,000*g* for 20 min and aliquots of the clear supernatant were assayed for PNMT using the technique described by Axelrod¹² and for tyrosine hydroxylase by the method of Nagatsu *et al.*¹³.

The adrenal weight decreased markedly in hypophysectomized rats (Table 1). After treatment with ACTH the adrenal weight was restored to the level found in control rats. After dibutyl cyclic AMP treatment there was only a small but significant increase in adrenal weight.

Adrenal PNMT levels decreased after hypophysectomy (Table 1) and were restored to almost control levels after treatment with ACTH. Dibutyl cyclic AMP treatment, however, resulted in only a small increase in PNMT levels, paralleling the increase in adrenal weight.

Adrenal tyrosine hydroxylase levels also decreased after hypophysectomy (Table 1) and were restored to control levels by ACTH treatment. Dibutyl cyclic AMP administration resulted in an increase in tyrosine hydroxylase to levels above those found in control animals.

Adrenal dopamine- β -hydroxylase levels also decreased after hypophysectomy (Table 2) and were restored almost to control levels by ACTH and dibutyl cyclic AMP treatment and to control levels by dexamethasone.

Table 2 Effects of ACTH and Dibutyl Cyclic AMP on Adrenal Dopamine- β -hydroxylase Activity in Hypophysectomized Rats

Group	Dopamine- β -hydroxylase activity (% of control value)
Sham hypophysectomized control	100.0 $\pm$ 5.6
Hypophysectomized	58.2 $\pm$ 4.8 *
Hypophysectomized + ACTH	86.4 $\pm$ 3.5 † §
Hypophysectomized + dexamethasone	102.3 $\pm$ 5.4 †
Hypophysectomized + dibutyl cyclic AMP	84.6 $\pm$ 4.0 † §

Three days after hypophysectomy rats were begun on 5 days of treatment with ACTH (5 IU subcutaneously/day), dexamethasone (1 mg subcutaneously/day) or dibutyl cyclic AMP (25 mg subcutaneously in 0.25 ml. 8% gelatin twice daily). Rats were killed 8 days after hypophysectomy. Levels of dopamine- β -hydroxylase in sham hypophysectomized controls were 3.76 $\pm$ 0.21 nmol octopamine/h/pair of adrenals. Results are expressed as means $\pm$ s.e. for groups of six to eight rats.

* $P < 0.01$ compared with sham hypophysectomized control group.

† Not significantly different from sham hypophysectomized control group.

‡ $P < 0.05$ compared with sham hypophysectomized control group.

§ $P < 0.01$ compared with hypophysectomized group.

|| $P < 0.001$ compared with hypophysectomized group.

The small but parallel increases in adrenal PNMT levels and adrenal weights after administration of dibutyl cyclic AMP may reflect a relatively small increase in steroidogenesis. Ney⁹ found a parallel between adrenal weight and ACTH-stimulated corticosterone secretion in hypophysectomized rats

treated with the same doses of dibutyl cyclic AMP for the five days beginning immediately after hypophysectomy. In our studies dibutyl cyclic AMP administration was begun 3 days after the operation. The small increase in PNMT levels after dibutyl cyclic AMP (Table 1) may be secondary to the production of only small amounts of glucocorticoids by the adrenal cortex. ACTH treatment caused a greater increase in adrenal weights and in adrenal PNMT levels. In the experiments where PNMT increased markedly in response to glucocorticoid administration the steroids were administered in very large doses^{1,2}.

PNMT levels are controlled primarily by the pituitary-adrenal system and only to a small extent by neuronal influences⁴. Dopamine- β -hydroxylase levels, on the other hand, are influenced markedly by both nerve impulses and the pituitary-adrenal system⁴. The more marked increase in dopamine- β -hydroxylase levels after treatment with dibutyl cyclic AMP (Table 2) is consistent with the view that nerve impulses influence levels of dopamine- β -hydroxylase through an effect on cyclic AMP in the adrenal medulla.

In hypophysectomized rats there is an increased turnover of noradrenaline in the heart¹⁴, suggesting an increase in nerve impulses. Nerve impulses to the adrenal medulla of hypophysectomized rats are probably also increased. This increase in nerve activity in hypophysectomized rats may make adrenal dopamine- β -hydroxylase more sensitive to low levels of glucocorticoids.

In normal rats the levels of cyclic AMP in the adrenal medulla are equal to those in the cortex (M. Paul *et al.*, unpublished observations). It is possible that administered dibutyl cyclic AMP acts directly on medullary dopamine- β -hydroxylase to increase levels of this enzyme. ACTH might directly cause an increase in medullary cyclic AMP or stimulate the secretion of glucocorticoids from the cortex which in turn might stimulate medullary cyclic AMP production; thus medullary cyclic AMP may act as a secondary messenger and cause an increase in adrenal dopamine- β -hydroxylase levels. It does not seem likely that changes in PNMT levels are caused by the direct action of dibutyl cyclic AMP or ACTH on the adrenal medulla.

Tyrosine hydroxylase levels are increased after ACTH treatment but not after glucocorticoids⁵; ACTH therefore probably does not act through glucocorticoids to increase the enzyme activity. After treatment with dibutyl cyclic AMP the marked increase in tyrosine hydroxylase activity, with only a small increase in adrenal weight, is probably secondary to a direct effect of the dibutyl cyclic AMP on the medulla. ACTH may directly stimulate medullary cyclic AMP which then acts as a secondary messenger to increase medullary tyrosine hydroxylase levels.

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Induction of Cleft Palate in Rats with Intra-amniotic Corticoids

TESTS of teratogenicity using tissue culture, laboratory rodents or primates may not yield results applicable to man¹. Different species and even strains differ in their response to teratogens. In mice, the percentage induction of cleft palate after administration of corticoids varies from strain to strain²; in rats, the effect cannot be found at all³⁻⁵. The relative influences of maternal metabolism on the one hand, and of specificity of the morphogenetic processes in the embryo on the other, remain unknown. The development of a test for the teratogenic effect of drugs depends on the solution of this problem: strict species specificity of the embryonic morphogenetic mechanisms would absolutely preclude extrapolation from laboratory animals to humans.

Administration of the substance to be tested directly into the amniotic sac of the embryo eliminates the influence of the maternal metabolism and of the placental barrier. We have therefore administered corticoids by this route, as described by Dostál⁶, to determine whether the resistance to cleft palate in rats is a function of the embryo or the mother.

Wistar-Konárove embryos were used. Doses of 0.2, 0.4 or 0.6 mg of soluble hydrocortisone (Spofa) dissolved in 3 μ l. of Ringer solution were injected into the amniotic sac on embryonic day 13, 14, 15 or 16. The results of the examinations on day 18 of embryonic development are presented in Table 1. After a single administration of various doses of hydrocortisone into the amniotic sac on day 14, 15 and 16 respectively the occurrence of cleft palate (CP) or of partial cleft palate (CPP)⁷ was recorded in twenty-one of forty-five embryos. Administration of an inert vehicle to forty-seven embryos in the same experimental conditions failed to produce any malformations of this type (Z. Rychter, R. J. and O. Marhan, in preparation).

Thus it can be concluded that hydrocortisone can induce cleft palate in rat embryos, provided that it is administered beyond

the placental barrier. This shows that the difference between the sensitivity of mouse and rat embryos to the teratogenic action of corticoids administered to pregnant animals should be sought in the transport channel before the placental barrier. It is not our aim to advance hypotheses about the possible causes of this resistance, but rather to draw attention to the value of intra-amniotic administration for the screening of drugs for teratogenic activity.

This experiment was initiated in Konárove in cooperation with RNDr. O. Marhan.

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Central Visual Discharge Time-locked with Spontaneous Eye Movements in the Cat

It is well known that there is an illusory displacement of the visual field in man when the eyes are moved passively, while active movement of the eyes results in a stable perception. The existence of a central neural mechanism as part of the excitation pattern of the overt movement has been postulated in an attempt to account for these phenomena^{1,2}.

Any efferent discharge, resulting in an ocular movement, would be accompanied by a concurrent central discharge into the visual system, the effect of which would be to "anticipate" and "counteract" those changes in afferent stimulation which result from the movement of the eyes (the "corollary discharge" of Sperry¹). Such a self-regulating compensatory mechanism would enable a constant visualized environment in normal conditions of active displacement of the eyeballs. If the eyes were moved passively, however, no concurrent central discharge would take place to compensate for the relative motion

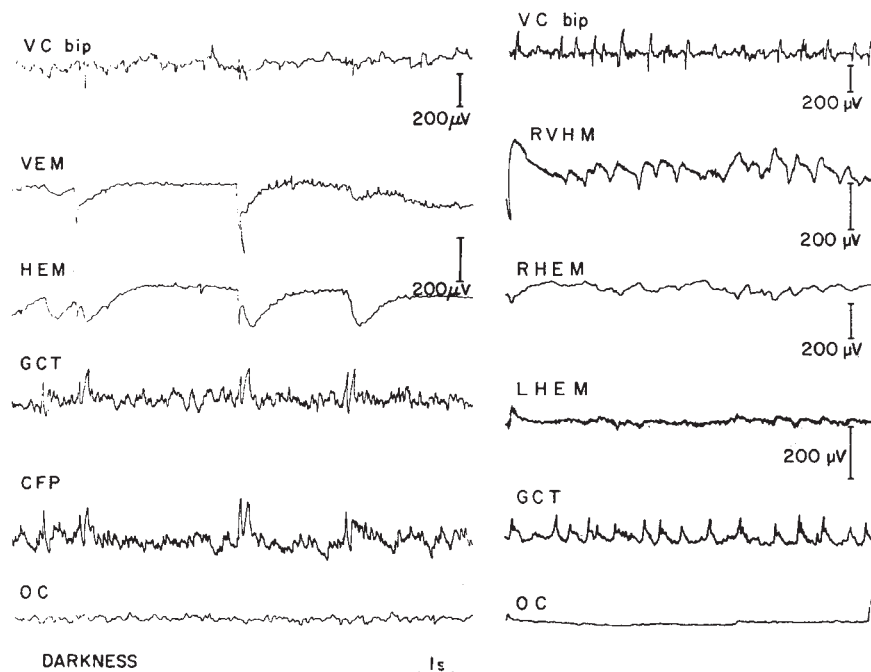
Table 1 Induction of Cleft Palate in Rat Embryos

Day number and dose	Normal		Treated embryos				Dead		Dead		Untreated embryos				Normal	
	No.	%	No.	CPP %	No.	CP %	No.	%	No.	%	No.	CP %	No.	CPP %	No.	%
13																
0.2 mg 5	17	77.3	1	4.5	0	0	4	18.2	2	8.7	0	0	0	0	21	91.3
14																
0.2-0.4* 4	1	7.1	6	42.8	5	35.7	2	14.3	0	0	0	0	0	0	20	100.0
15																
0.6 mg 5	2	12.5	3	18.7	3	18.7	8	50.0	0	0	0	0	0	0	12	100.0
16																
0.6 mg 4	10	66.7	3	20.0	1	6.7	1	6.7	0	0	0	0	1	6.7	14	93.3

Hydrocortisone soluble Spofa injected into amnion of Wistar-Konárove rats. Controls were taken from the opposite horn of the same female.

* Ten embryos were injected with 0.2 mg, four embryos with 0.4 mg.

Fig. 1 Two examples of phasic changes of the integrated activity of the dark discharge time-locked with eye movements in the central visual pathways, with no change occurring at the optic chiasm. Two different experiments. Note the wave patterns developing simultaneously at the visual cortex. VC, Visual cortex; VEM, vertical eye movements; HEM, horizontal eye movements; GCT, geniculo-cortical tract; CFP, cortico-fugal pathway; OC, optic chiasm.



of objects on the retina, with a consequent illusory movement of the surround³.

If valid, these models might account for various related phenomena, such as the suppression of blurring of vision between fixations in eye movements⁴, the reduction of visual performance⁵ and responsiveness⁶⁻⁹ during saccades; binocular rivalry⁶; some spontaneous optokinetic responses produced by visual inversion¹⁰, and so forth. Indeed, as Sperry¹ wrote "we may imagine the background excitatory state of the visual brain field undergoing continuous fluctuation of an overall gradient type in response to any movement or shift of posture that affects the direction of gaze".

A plausible electrical index of the background excitatory state of the visual system may be represented by the maintained spontaneous discharge ("the energizer" of Granit¹¹) present even in darkness along the entire visual pathways. Therefore, if the postulates were correct, we should expect to find transient changes in the activity level of the dark discharge, time-locked with active eye movements, only at the central sites of the visual system. No such phasic changes should occur in the activity level of the optic chiasm, and none should be found anywhere during passive eye movements.

We have performed experiments designed to verify these

assumptions. Thirty-four adult cats were used in an "écephale isolé" preparation, the classical Bremer's transection which makes possible the study of numerous spontaneous eye movements. The spinal transection was performed under short-acting barbiturate (sodium methohexital, 7 mg/kg given intravenously) or ether anaesthesia. After transection, the general anaesthesia was discontinued and the animals were artificially respired and warmed. Thorough infiltration with 2% xylocaine was performed repeatedly throughout the experimental session at all pressure points of the stereotactic device, and at all operative sites to avoid every source of pain. The levels of electrical activity from a large population of fibres in the optic chiasm (OC), in the geniculo-cortical tract (GC), and in a non-commissural cortico-fugal (CF) pathway were measured in dark adaptation. The effectiveness of separating the ascending fibre tract (output from the lateral geniculate body) from the descending tract (output from the visual cortex) has been ascertained both anatomically¹² and electrophysiologically¹³.

The recording techniques, as well as the methods for integration of the electrical activity from a large population of fibres, followed the description of Arduini and Pinneo¹⁴. Briefly, the activities recorded from bipolar electrodes were amplified and

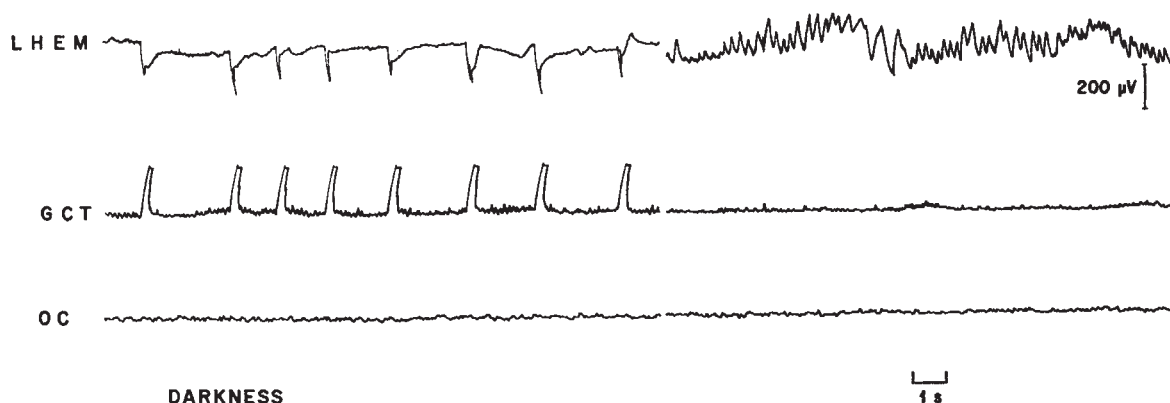
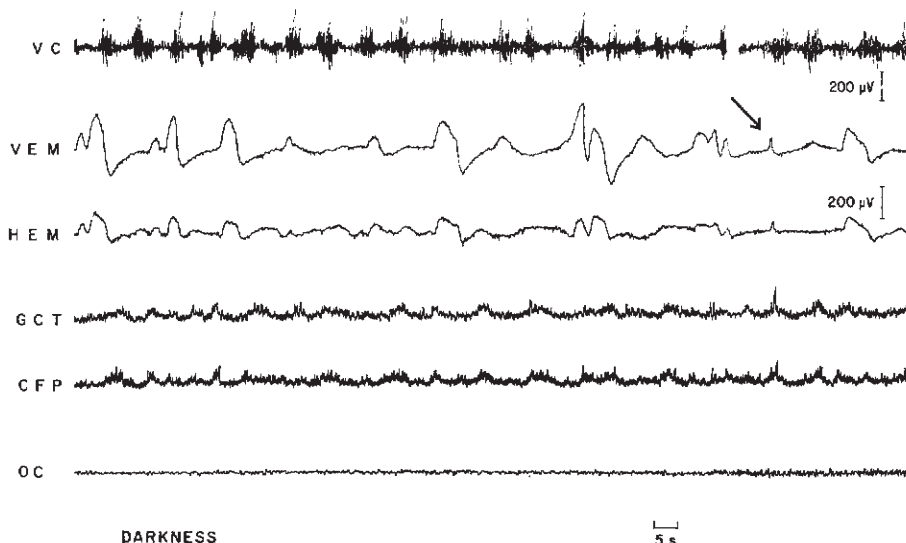


Fig. 2 At the left, a sample of active, rapid eye movements time-locked with phasic changes in the level of the dark discharge at the geniculo-cortical tract with no changes at the chiasm. At right, a sample of rapid, and slower, passive eye movements not accompanied by changes in the rate of firing at either tract. Abbreviations are as in Fig. 1.

Fig. 3 An illustration of the need for fast spontaneous eye movements to elicit the increased firing rate of the central visual pathways. Note that while slower, drifting eye movements are ineffective, a small rapid movement, at the right of the figure (arrow), shows a phasic change in the level of the dark discharge at both central tracts. The fluctuations in GCT and CFP are linked to the spindle-bursts at the visual cortex (synchronized state). Abbreviations are as in Fig. 1.



deprived of low frequency components. The filtered signals were rectified and fed into an integrator system. In several experiments, both integrated as well as mass activity were recorded simultaneously. The electrocorticogram (ECG) was monitored by means of bipolar silver-silver chloride ball electrodes placed 2 mm apart on the visual cortex. The eye movements (EOG) were recorded by means of two orthogonally placed pairs of silver-silver chloride electrodes, across one or both eyes in the vertical and horizontal planes, and derived through d.c. coupling amplifiers. Artefacts due to blinking were monitored by needle electrodes inserted at the base of the lids. All the electrical activities recorded were monitored, together with the electrokinetogram (EKG), on a strip-chart through a Grass model 7 polygraph. The mass activity recorded from the three visual pathways, the ECG, the EOGs were simultaneously stored on magnetic tape for later analysis. Passive eye movements were obtained mechanically by means of silk threads encircling the isolated external rectus or superior oblique muscles of the eye. In several animals curarization was induced with intravenous 'Flaxadyl' (2-5 mg/kg). The exact positions of the depth electrodes were checked by serial section histology (LFB methods). Each experimental session began after at least 30 min of total dark adaptation, and at least 2 h after cessation of general anaesthesia.

Our results show phasic changes in the level of the integrated dark discharge in temporal relation to spontaneous eye movements that occurred only in the GC and in the CF pathways, while no change was seen at the OC (Fig. 1).

Specifically, we observed the following. (1) Concomitant with spontaneous eye movements, the activity level of the central dark discharge was consistently and substantially augmented only in the central visual pathways (GC and CF tracts), with no change in the level of the discharge at the input to the lateral geniculate body (LGB). (2) Passive eye movements were not accompanied by changes in the level of the dark discharge at either the OC or at the central visual pathways (Fig. 2). (3) Although there seemed to be no direct relationship between the amplitude of the eye movement and that of the phasic increase in central visual activity, to obtain the latter, certain characteristics of the ocular movements must be present: (a) the eye movements time-locked with the phasic increase in central visual firing rate must be conjugate and (b) only eye movements with an EOG fast rising phase are associated with the enhancement in central visual impulse traffic (Fig. 3). (4) Concomitant with the phasic increase of discharge in the central visual pathways during the eye movements which we have described, we observed a simultaneous cortical wave in the ECG (Figs. 1 and 4). (5) The paralysis of extrinsic eye muscles by curarization did not abolish either

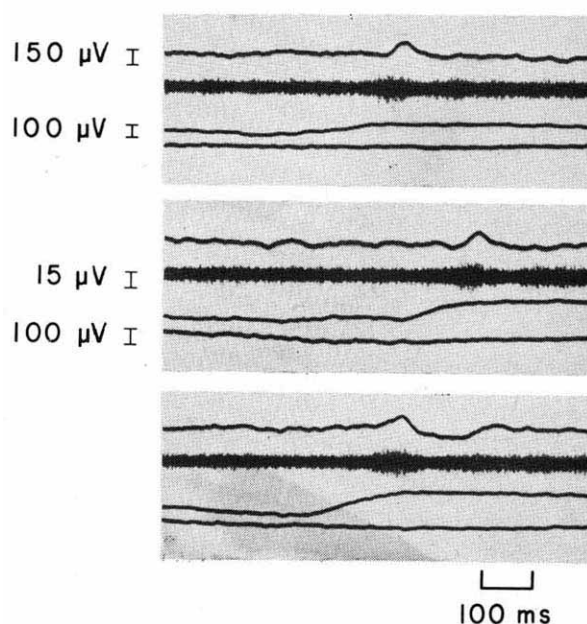


Fig. 4 A photo strip to illustrate changes in mass activity recorded at the GCT, time locked with rising phase of an eye movement. Note the concomitant cortical wave developing. The first trace in each strip is the ECG. The second is the fibre mass activity derived from the GCT. The third and fourth are the EOG, horizontal and vertical respectively.

the phasic increase of discharge in the central visual pathways, or the concomitant wave derived from the visual cortex.

Our results, therefore, seem to provide electrophysiological evidence to support the postulate of a central "corollary discharge" time-locked with active movements of the eyes. Waves similar to those we obtained from the visual cortex, in association with active eye movements, have been observed in the LGB and visual cortex of chronic, awake animals during eye movements (cat¹⁵, monkey¹⁶, and rabbit⁹). The perfect temporal correspondence we observed between the phasic increases of the central dark discharge and the waves detectable at the cortex also supports the hypothesis that the wave patterns previously detected in the LGB and visual cortex during rapid eye movement sleep^{17,18}, and related to a phasic change in LGB neuronal firing rate¹⁹, cannot be related only to the mechanisms of the visual imagery during dreaming²⁰. Rather, those LGB and visual cortex wave patterns seem to represent a reliable electrical signal of visuo-motor integration.

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Corticotrophin Releasing Factor in Hypophysial Portal Blood of Rats

IN spite of the work on factors in brain tissue which seem to release pituitary hormone¹, little has been done to determine whether these factors are present in hypophysial portal blood. Early work² suggested that blood from the empty sella turcica of hypophysectomized dogs can stimulate the release of adrenocorticotrophic hormone (ACTH) and, more recently, it has been shown that there is luteinizing hormone releasing activity in hypophysial portal blood of rats^{3,4}. We have now investigated whether hypophysial portal blood of rats contains a corticotrophin releasing factor (CRF).

Blood was collected⁵ from the cut pituitary stalk of adult male Wistar rats and corticotrophin releasing activity was assayed by injecting the substances to be tested intravenously into adult male Wistar rats which had been treated with chlorpromazine, morphine sulphate and 'Nembutal'⁶. The assay animals were exposed to an atmosphere of 95% O₂ and 5% CO₂ at frequent intervals during anaesthesia. Twenty minutes before injection of the test substance, approximately 2 ml. of blood was withdrawn as a pre-treatment sample from the external jugular vein. A post-treatment sample of blood (3–5 ml.) was obtained from the abdominal aorta 15 min after injection of the test substance. The concentration of corticosterone in the plasma of the pre- and post-injection samples was assayed by the method of Mattingly⁷. An increment in plasma corticosterone indicated that the test substance contained ACTH or that it was capable of causing the release of endogenous ACTH. To determine the proportion of the increment attributable to corticotrophin releasing activity, the substances were also injected into rats which had been hypophysectomized 8–13 h before and which had received injections of chlorpromazine and 'Nembutal', but not morphine sulphate.

Corticotrophin releasing activity was determined in untreated hypophysial portal plasma and in acid ethanol extracts of portal plasma which were obtained as described previously⁴. The extract of plasma was ultrafiltered at 50 pounds/inch² using a 'Diaflo' (Amicon) apparatus with a UM-10 filter (presumed exclusion limit, molecular weight of 10,000), and the residual, non-diffusible material and filtrate were lyophilized and stored at –15° C. Immediately before assay, the residue and filtrate were dissolved in 0.9% saline, neutralized with concentrated NaOH and centrifuged at 24,000g for 30 min to sediment a fine precipitate. As Table 2 shows, injection of either untreated hypophysial portal plasma (experiment 2; dose equivalent to 2 h collection per rat) or the residue of extracted portal plasma (experiments 4 and 5; dose equivalent to 4 h collection per rat) was accompanied by a considerable increase in plasma corticosterone in the assay animals. In each experiment, however, the mean of the increments in intact rats was significantly greater than that in hypophysectomized animals. Neither the administration of the ultrafiltrate of extracted hypophysial portal plasma nor that of the residue or filtrate of extracted systemic plasma (experiment 3; volume injected per rat equivalent to dose of extracted portal plasma) was followed by an appreciable change in the concentration of plasma corticosterone. These results suggest that untreated and acid ethanol extracted hypophysial portal plasmas contain a factor which causes the release of ACTH. Detectable quantities of this factor were not present in an extract of systemic plasma. The differences in the levels of plasma corticosterone in the two types of assay animals cannot be attributed to a reduced sensitivity of the adrenal cortex to corticotrophin in the hypophysectomized group because, in preliminary studies (Table 1), the sensitivity of the hypophysectomized rats to ACTH was similar to or higher than that of the intact animals.

Table 1 Sensitivity of Assay Animals to ACTH

Treatment	Increment in corticosterone (µg/100 ml. plasma)	
	Intact	Hypophysectomized
5 mU ACTH	13.8 ± 3.0 (3) *	11.1 ± 3.2 (3)
25 mU ACTH	15.1 ± 2.0 (6)	29.9 ± 2.1 (3)
50 mU ACTH	21.8 ± 3.3 (5)	32.9 ± 7.2 (3)

* Mean ± s.e. (number of animals).

Various doses of porcine ACTH were injected intravenously into either intact (treated with chlorpromazine, morphine sulphate and Nembutal) or hypophysectomized (treated with chlorpromazine and Nembutal) rats. The increments in plasma corticosterone which followed the injection of ACTH in hypophysectomized rats were similar to or higher than those in intact animals, suggesting that, in these conditions, the sensitivity of the adrenal cortex to ACTH was not impaired by hypophysectomy.

The increase in plasma corticosterone in hypophysectomized animals (experiments 4 and 5) indicated that ACTH was also present in hypophysial portal plasma. To check the results of the differential bioassay method which we used, the ACTH in portal plasma was inactivated by the method of Geschwind and Li⁸. Sodium metaperiodate (0.5 m) was added to ice-cold hypophysial portal plasma to make a final concentration of 0.1 M. The mixture was shaken at room temperature and, after 8 min, the reaction was stopped by the addition of excess glucose. The mixture was then extracted with acid ethanol⁴. Immediately before assay, the extract was dissolved in 0.9% saline, neutralized with concentrated NaOH and centrifuged at 45,000g for 30 min to sediment the precipitate. The injection of the supernatant (dose equivalent to 4 h collection per animal) was followed by a marked increase in the concentration of plasma corticosterone (experiment 6) in the intact animals but it did not seem to affect the level of corticosterone in hypophysectomized rats. This result suggests that periodate oxida-

Table 2 Assay of Hypophysial Portal Plasma for Corticotrophin Releasing Activity

Experiment	Treatment	Intact	Increment (μ g corticosterone/100 ml. plasma) Hypophysectomized	Difference	Significance
1	Saline	-2.5 ± 1.5 (5)*	-2.8 ± 1.7 (5)	-0.3	NS
2	Portal plasma	37.3 ± 3.9 (5)	20.8 ± 2.6 (3)	16.5	$P < 0.01$
3	Acid ethanol extract of systemic plasma				
	residue	-1.3 ± 1.9 (6)	-0.9 ± 0.4 (3)	-0.4	NS
	filtrate	-0.7 ± 1.2 (3)	2.2 ± 1.9 (3)	2.9	NS
4	Acid ethanol extract of portal plasma				
	residue	52.9 ± 2.1 (4)	32.5 ± 5.1 (5)	20.4	$P < 0.001$
	filtrate	-4.9 ± 1.6 (3)	-0.2 ± 1.3 (3)	-4.7	NS
5	Acid ethanol extract of portal plasma				
	residue	40.1 ± 1.5 (6)	28.3 ± 2.9 (5)	11.8	$P < 0.01$
	filtrate	-4.6 ± 1.1 (4)	-2.2 ± 0.5 (4)	-2.4	NS
6	Acid ethanol extract of sodium periodate treated portal plasma	40.4 ± 1.2 (4)	-0.5 ± 2.1 (4)	40.9	$P < 0.001$

* Mean $\pm$ s.e. (number of animals).

Significance of difference (increment in intact versus increment in hypophysectomized rats) determined by unpaired Student's *t* test.

tion reduces the activity of ACTH in hypophysial portal plasma but that it has no significant effect on the potency of CRF.

An explanation for the fact that neither ACTH nor CRF seemed to pass across the filter could be that their molecular weight in hypophysial portal blood is greater than 10,000. A more likely explanation would be that these substances were bound either to plasma proteins or to the membrane. In contrast to these findings, Fink, Lee and Burger⁹, using similar techniques, have demonstrated the presence of luteinizing hormone releasing activity in the ultrafiltrate of extracted portal plasma, and it is significant that Porter and Rumsfeld² found that the CRF activity of canine hypophysial portal blood resides in a lipoprotein-rich fraction. This activity disappeared after dialysis.

It is well known that vasopressin can stimulate the release of ACTH¹⁰, but the absence of pressor activity in rat pituitary stalk blood³ and the finding that antidiuretic activity of fluid washings of the rat pituitary stalk collected over 1 h is equivalent to only 66–98 nU of arginine vasopressin¹¹ suggest that the quantity of vasopressin in hypophysial portal blood is insufficient to account for our results.

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Oxytocin Antibody and Lactation and Parturition in Rats

To show that a hormone is essential to a particular physiological process it is best to study an isolated deficiency state, but this has so far been difficult for oxytocin. Hypophysectomy, lesions in the hypothalamus and the administration of drugs—the experimental methods used so far—cannot produce a unihormonal deficiency with certainty, with the result that there persist differences of opinion about the role of oxytocin in the initiation and maintenance of lactation and parturition^{1,2}. Oxytocin antibody of high titre and affinity³ provides, however, a new tool for these studies.

We have used primiparous mature female Sprague-Dawley rats, fed Purina chow and water and kept at $78 \pm 2^\circ$ F. Males and females were kept together, and the day in which sperm were found in the vaginal smear was taken as the day of mating.

On the twentieth day of pregnancy, each of eight rats was injected intraperitoneally with 1 ml. of pooled rabbit antiserum (oXy-AB) to synthetic oxytocin (Parke-Davis), and subsequently injected with 0.5 ml./day until parturition. Seven control rats were injected with normal rabbit plasma on a similar schedule. On the expected day of parturition the rats were inspected frequently. The litters were weighed at birth and each morning for four consecutive days, and stomachs were visually inspected for milk content daily.

In a separate experiment normally lactating rats were injected intraperitoneally on the tenth day of lactation with 1 ml. of pooled rabbit antiserum to synthetic oxytocin. Control lactating rats were injected with normal rabbit plasma. The pups were subsequently examined and weighed daily for 7 days.

The plasma of the recipient rats drawn 3 days after the injection of antibody, bound more than 75% of ¹³¹I-oxytocin at a 1:100 dilution, as measured by a double antibody technique³.

The mean gestation period for the treated rats was 22.1 ± 0.2 days compared with 21.9 ± 0.3 days in the control group, the difference not being statistically significant (Table 1). No obvious difficulties with delivery were noted, but ten of seventy-four pups were found dead shortly after birth in the treated group, compared with two of sixty-nine in the control group ($P < 0.10$).

To match litter size in the control group with that in the treatment group, several litters were immediately excluded from both groups, leaving five mothers in each group with litters of identical size for treatment and control regimens. Forty-nine

pups were studied in each group. Although the weight of pups did not differ significantly at birth, mean weight was significantly reduced in the treatment group on days 2, 3 and 4 (Table 2); furthermore, much less milk was observed in the transparent stomachs of the pups in the treatment group.

In animals injected on the tenth day of lactation, after normal lactation and pup growth had been maintained for some time, litter growth stopped for 1 day after injection of antibody and did not return to normal until the third day after injection (Table 3).

Table 1 Effect of Oxytocin Antibody on the Length of Gestation in Rats

Day of gestation	No. of animals giving birth on gestation day Oxytocin antibody (8)	Control (7)
21	0	2
22	7	4
23	1	1
	Mean 22.1 ± 0.2 days	Mean 21.9 ± 0.3 days

Numbers in parentheses refer to the number of rats per group.

Table 2 Effect on Pup Weight of Oxytocin Antibody injected into Pregnant Rats

	Weight of pups (g) during first 4 days of life ± s.e.			
	Day 1	Day 2	Day 3	Day 4
Control (49)	5.5 ± 0.1	6.2 ± 0.3	7.4 ± 1.3	9.0 ± 1.5
Oxytocin antibody (49)	5.3 ± 0.4	5.3 ± 0.6	5.9 ± 0.8	6.9 ± 1.2
<i>P</i>	<0.2	<0.05	<0.05	<0.05

Numbers in parentheses refer to number of pups in each group. *P* value obtained by Student's *t* test.

Table 3 Effect on Pup Weight Gain of Injection of Oxytocin Antibody on Tenth Day of Lactation

Days of lactation	Control (7) Mean ± s.e.	Treatment (8) Mean ± s.e.	<i>P</i>
9	14.2 ± 1.0	13.5 ± 1.1	NS
10	15.1 ± 1.1	14.0 ± 1.7	NS
11	13.1 ± 2.1	-0.5 ± 2.9	<0.005
12	12.9 ± 1.6	4.5 ± 2.9	<0.025
13	13.1 ± 1.1	12.0 ± 1.5	NS
14	14.9 ± 1.6	16.8 ± 1.5	NS
15	13.9 ± 1.4	9.8 ± 2.2	NS
16	17.3 ± 1.8	16.4 ± 2.4	NS
17	16.1 ± 2.3	14.9 ± 2.7	NS

Numbers in parentheses refer to the number of litters per group. *P* value obtained by Student's *t* test, for difference between treatment group and control group. NS, Not significant (>0.05).

The antiserum used was of high titre, was capable of neutralizing oxytocin in *in vitro* bioassays* and had a high degree of specificity for oxytocin³, with a cross reactivity of less than 1% for arginine vasopressin. Its unequivocal effects on the initiation and maintenance of lactation in rats provide conclusive evidence that the secretion of oxytocin is essential to both processes. This conclusion confirms most current thinking on this topic^{1,4}.

* The antiserum neutralized oxytocin in an *in vitro* mammary gland bioassay in our laboratory, and in an *in vitro* rat uterus assay in the laboratory of Dr Wilbur Sawyer.

On the other hand, the failure of antibody injection to affect parturition leaves the role of oxytocin in normal parturition much less clear. Because lactation was impaired in the same animals in which parturition proceeded on schedule and in which high titres of circulating antibody had been observed, this cannot have been the result of antibody titres too low to bind most of the circulating oxytocin. Several interpretations of the data are possible. (1) Differing sites on the oxytocin molecule may be responsible for milk ejection and uterine muscle contraction, with the antibody directed primarily against the former site⁵. But the successful neutralization by the antibody of the biological activity in both uterine muscle and mammary strip bioassays³ demonstrates that our antibody is directed against both sites. (2) The pattern of oxytocin secretion and of target tissue response may be different for milk ejection and uterine contraction. Thus, if parturition were associated with a spurt-like pattern of oxytocin secretion and lactation were not, enough oxytocin might well reach uterine receptors to activate contraction before the antibody could bind the oxytocin, while the postulated slower increase of oxytocin concentrations with lactation might be neutralizable by antibody. Alternatively, uterine receptors might have a higher affinity for oxytocin than those of mammary tissue. No convincing evidence has been presented for either of these hypotheses. (3) Finally, it is possible that oxytocin is not essential for parturition in rats. It may nevertheless aid the process, and our observations concerning the date of parturition do not exclude this possibility. Indeed, the greater occurrence of foetal death in litters of mothers receiving antibody supports the notion that parturition may have been abnormal in these animals.

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Control of Water Movement in Flounder Urinary Bladder by Prolactin

THE role of the amphibian urinary bladder in osmoregulation is well documented^{1,2}. In fish, however, maintenance of water and ion balance has been attributed almost entirely to the gill, kidney and intestine. Modification of ionic composition has been reported in the bladder urine of *Lophius americanus*³, *Platichthys flesus*⁴ and *Opsanus tau*⁵, and there has been one report of a transient effect of arginine vasotocin on water reabsorption from the bladder of *Tilapia mossambica*⁶; there

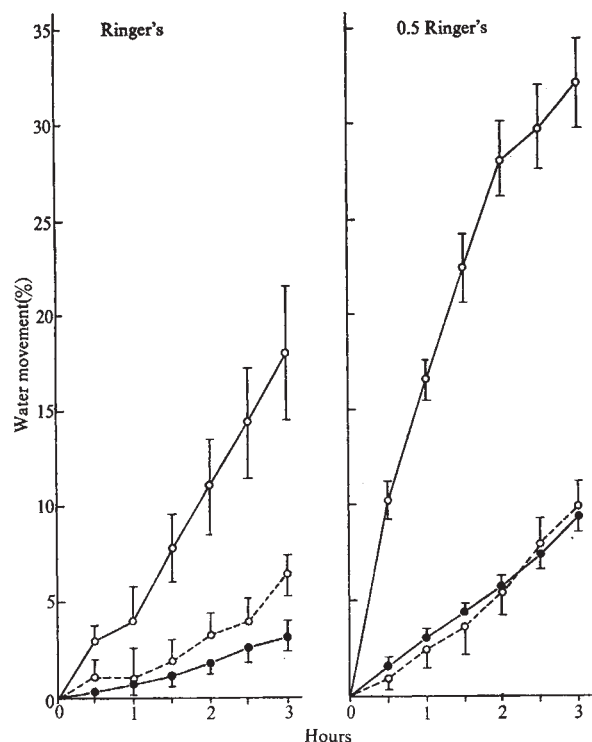


Fig. 1 Comparison of water movement out of isolated urinary bladders of *Platichthys stellatus* taken from 133% sea water (○—○) and fresh water (●—●) and the effect of prolactin (○- -○). Note increased rate of movement when bladder contains hypotonic Ringer solution. Vertical bar shows standard error of the mean ($n=5$).

has, however, been no direct physiological evidence of osmoregulatory activity of the teleost urinary bladder.

We have investigated this question using isolated bladders from the euryhaline flounder, *Platichthys stellatus*. Fish of both sexes, weighing about 200 g, were collected from estuarine water, which varied from dilute (15%) to concentrated (116%) sea water. They were kept for at least 7 days in 33% sea water, from which they were transferred to either fresh water (FW) or concentrated (133%) sea water (CSW) and kept for a minimum of 7 days before use. Some CSW-adapted fish were injected intraperitoneally with 1 mg of ovine prolactin (NIH PS-6) daily for 6 days and killed by decapitation 24 h after the last injection.

After the bladder had been separated from the peritoneum, the mesonephric duct anterior to the bladder was tied off with cotton thread, and a flanged capillary tube was inserted and tied in place near the cloacal end; the bladder was then removed. The isolated bladder was rinsed and filled with Ringer solution (150 mM NaCl, 2.5 mM KCl, 3.5 mM CaCl_2 , 1.0 mM MgCl_2 , 7.0 mM NaHCO_3 , 0.7 mM NaH_2PO_4 , 0.05% glucose, pH adjusted to 7.4 with HCl), and then immersed in the same solution and allowed to equilibrate for 30 min at about 20° C. The bladder was next filled with either isotonic or 20% Ringer solution until just distended (usually 0.4–0.5 ml.), and the tip of the capillary tube was sealed. These two concentrations of Ringer were introduced because urine osmolality of CSW fish is only slightly hypotonic to the plasma, whereas that of flounder in fresh water is about 15% of the plasma. The filled bladder was lightly blotted at the bottom with filter paper, weighed and transferred to 15 ml. of aerated Ringer solution. Weights were recorded at 30 min intervals, and the bathing Ringer solution was changed hourly. After 3 h, the internal (mucosal) solution was removed, and the empty bladder was weighed to determine the initial volume. Water movement is expressed as the percentage of water lost from the mucosal (lumen) side (Fig. 1). Expression of the

data in subsequent experiments as $\mu\text{l./cm}^2/\text{h}$ indicated no difference in the comparisons presented.

Bladders isolated from CSW flounder and bathed on both mucosal and serosal sides with isotonic Ringer solution have a significantly higher rate of water movement than those from FW fish. Water loss from the mucosal side was 18% and 3%, respectively, for the 3 h of incubation. The difference was more marked when bladders were filled with 20% Ringer, with higher rates of water movement in both groups.

Prolactin treatment of CSW fish caused their bladders to behave as if they had been taken from fish in fresh water, a habitat which stimulates a high level of prolactin secretion⁷. Bladders of CSW fish treated *in vivo* with prolactin showed a lower rate of water movement than in bladders from CSW controls, comparable with that seen with bladders from FW fish, whether maintained in the presence or absence of an osmotic gradient. A critical role for prolactin in teleost osmoregulation, at the branchial and renal levels, is generally accepted^{7–10}. Inhibition of permeability to water by prolactin has been seen with isolated gill (*Gasterosteus*)¹¹ and intestine (*Anguilla*) (unpublished observations of T. H.) preparations. Urine flow decreased in hypophysectomized (hence prolactin-devoid) *Carassius*¹³, while prolactin treatment increased urine flow in hypophysectomized *Carassius*¹³ and *Fundulus*¹⁴. Prolactin also had a diuretic effect on intact *Gasterosteus*¹⁵. Although these observations may not reflect bladder function, they are consistent with our finding of inhibition by prolactin of water movement across the bladder wall.

In the urinary bladder of CSW flounder, water movement occurred in the absence of an osmotic gradient. A possible explanation of water movement in the absence of a gradient is that ion transport across the cell membrane may "drag along" water molecules². In our *in vitro* preparation with isotonic Ringer solution inside, net effluxes of sodium (mucosa to serosa) were consistently observed. The sodium concentrations of the fluid passing across the membrane were higher in all cases (FW 1,370 mequiv/l.; SW/prolactin 510 mequiv/l.; SW 180 mequiv/l.) than that of the introduced Ringer solution (150 mequiv/l.). Preliminary investigation indicates that water movement is closely related to active transport of sodium. It seems probable therefore that in sea water the flounder bladder reabsorbs water from the urine by actively transporting sodium, whereas in fresh water it transports sodium in the presence of a permeability barrier which interferes with water movement. The same type of modification of ionic concentration occurs in the proximal tubule of the kidney¹⁶, probably more effectively than in the bladder. It is pertinent to note that the teleost urinary bladder is formed by an enlargement of the mesonephric ducts¹⁷.

Our results indicate that the teleost urinary bladder, at least in the starry flounder, may play an important role in osmoregulation by modifying the concentration of the urine, and could thereby make a contribution to hydromineral balance more important than mere storage and expulsion of urine.

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Possible Hepatic Function for Crustacean Blood Cells

CRUSTACEAN blood is generally considered to function as a transport medium for low molecular weight metabolites. Conversely, the hepatopancreas has been regarded as a liver analogue—the principal site for the storage and metabolism of fats and carbohydrates¹. It now seems likely that the blood tissue is as important as the hepatopancreas for the storage and metabolism of polysaccharides.

Whole blood (haemolymph) and homogenates of hepatopancreas from two species of decapod crabs, *Libinia emarginata* and *Carcinus maenas*, were treated with 80% ethanol to separate the oligosaccharides from the ethanol-insoluble polysaccharides. Precipitated polysaccharides were resuspended in water and subjected to acid hydrolysis. Qualitative separation of the sugars from both fractions was accomplished by thin-layer chromatography². In the oligosaccharide fraction of both tissues, glucose, maltose, fructose, trehalose and fucose were recovered consistently. The hydrolysates of the polysaccharide fraction contained glucose, maltose, fructose and glucosamine.

Quantitative assays by the methods of du Bois *et al.*³ showed a marked difference between the two tissues in the distribution of oligosaccharide and polysaccharide groups (Fig. 1). Surprisingly, the blood was found to contain large amounts of polysaccharide. Oligosaccharides, however, predominate in the hepatopancreas, no more than 10–20% of the carbohydrate occurring as polysaccharide. Calculations of the total tissue carbohydrate underline this disparity in polysaccharide content. The total tissue polysaccharide was estimated by determining the mean blood volume and mean weight of hepatopancreas. In fifty *Carcinus* (mean body weight, 84.1 ± 3.1 g) the haemolymph contained a total of 12.0 ± 0.6 mg of polysaccharide whereas the hepatopancreas had only 3.4 ± 0.6 mg. Thus, not only is the proportion of polysaccharide higher in blood tissue, but the total amount, in comparison with the hepatopancreas, is also greater in haemolymph.

Acid hydrolysis of the haemolymph polysaccharide from *Carcinus* yields approximately 25% of the total concentration as free glucose, the remainder comprising large amounts of glucosamine, with maltose and fructose appearing in smaller quantities. Johnston and Fisher⁴ have shown that up to 60%

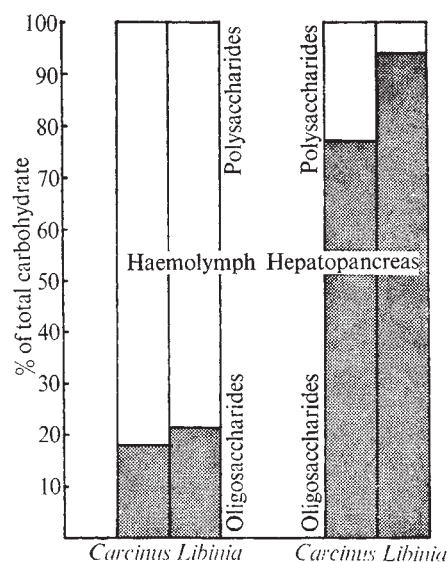


Fig. 1 Histograms to compare the composition of the carbohydrates of the haemolymph and the hepatopancreas of the two crabs *Carcinus* and *Libinia*. Oligosaccharides are shaded.

of the blood polysaccharide from *Libinia* is also recovered as glucose on hydrolysis. These results strongly suggest that glycogen is an important component of the carbohydrates in circulation. Since glycogen is not normally transported in the plasma fraction, further examination of the blood of *Carcinus* was carried out in an attempt to localize the glycogen complex. Analysis of blood which has been gently centrifuged (500–1,000g for 5 min) to remove cellular elements showed that glucose is absent from the hydrolysed polysaccharide fraction of the plasma but is present in the cell fraction. Histochemical examination (discussed later) of *Carcinus* haemocytes confirmed that the blood glycogen is an intracellular carbohydrate.

Table 1 Histochemical Tests on *Carcinus* Haemocytes

Histochemical test	Cell type	
	A	B
The PAS reaction	+	+
Diastase extraction and PAS	—	+
Alcian blue staining	—	—
Hale's dialysed iron test	—	+

Four morphologically distinct types of blood cell were found and a detailed cytological description of them will be published elsewhere. In only two of the four types were significant deposits of carbohydrate detected by the tests used (Table 1). Type A cells are strongly PAS positive although staining is eliminated if the cells are treated with salivary amylase beforehand. Type A haemocytes gave negative results with Hale's dialysed iron test and with Alcian blue. It would seem, therefore, that glycogen is the principal cellular polysaccharide. Type B cells remain PAS positive after diastase extraction, however, and are also positive to Hale's test. While glycogen may be present in these cells, there is also a significant amount of non-glycogen acid polysaccharide. The presence of glycogen has also been confirmed by electron microscopy. As with light microscopy, not all the haemocytes are found to contain glycogen. Many cells, however, contain masses of cytoplasmic glycogen recognizable by its characteristic granular size—approximately 20 nm—and dense staining with lead (Fig. 1).

Although this evidence suggests that the haemocytes of *Carcinus* maintain stores of both glycogen and non-glycogen polysaccharides, one can only speculate on the relative importance of the cells in the storage of total body polysaccharide

material. Both muscle and epidermal tissues are known to be sites for glycogen deposition⁵, but their role in carbohydrate metabolism has not been fully investigated in the Crustacea. It does seem, however, that the haemolymph assumes a function, quantitatively more important than that of the hepatopancreas, in the synthesis and storage of polysaccharides.

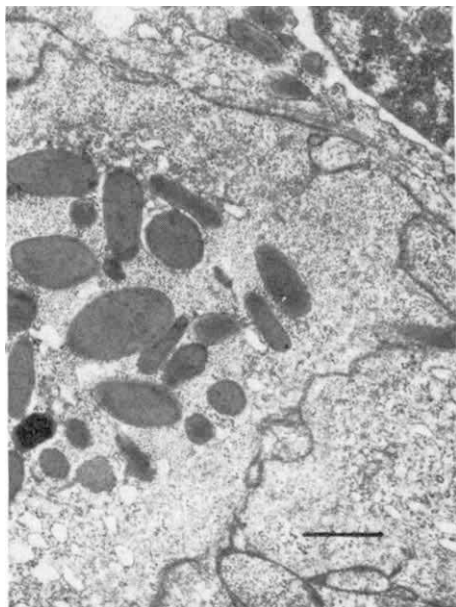


Fig. 2 Electron micrograph showing parts of several cells from an aggregate made by lightly centrifuging *Carcinus* haemolymph, fixing in glutaraldehyde in phosphate buffer (pH 7.4), post osmication and lead staining. Most of the cytoplasm is packed with glycogen granules in the β form; in some cells the α form is also seen. Smooth endoplasmic reticular vesicles are frequently associated with the glycogen deposits. A number of large, unidentified, dense staining vesicles are also prominent. The marker is 1 μ m.

The presence of glycogen in the haemocytes raises some interesting possibilities, particularly because many of the electron micrographs show extensive packing of the cytoplasm with the glycogen granules. It is difficult, from comparison with mammalian systems, to conceive of the carbohydrate acting solely as an energy reserve for the basic metabolic functions of the cells. It is possible that these cells may, as in mammalian liver cells, have the ability to break down the glycogen to glucose which would be liberated. The prerequisite for such glycogenolysis is the presence of the enzyme glucose-6-phosphatase. Preliminary results from both biochemical and histochemical analyses indicate that *Carcinus* haemocytes do contain G-6-phosphatase.

Our evidence clearly shows that in these decapods, cells of the haemolymph maintain large quantities of polysaccharide, and that a large part of this is in the form of glycogen. The possibility that these cells act rather like a liver tissue, liberating free hexose sugars to circulation, is under investigation.

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Production of an Insect Sex Attractant by Symbiotic Bacteria

MALES of the grass grub beetle *Costelytra zealandica* (White) (Coleoptera : Scarabaeidae), the principal insect pest of pastures in New Zealand, are attracted to an adhesive preparation¹ and to a resin² both of which seem to contain a chemical sex attractant. These materials contain small amounts of free phenol, which has been shown to be attractive to male beetles and seems to be the only attractant present in the resin. Furthermore, phenol may be the natural attractant of *C. zealandica*^{3,4}. Most studies of structures associated with the production of insect sex pheromones have been concerned with the Lepidoptera, and in spite of increasing interest in pheromones produced by the Coleoptera, little has been published. We have attempted to locate possible sites of pheromone production in the female grass grub beetle.

We investigated the colleterial glands of *C. zealandica*, which lie beneath the seventh sternite ventral in position to the vagina. They are paired and oval shaped, each consisting of two rounded lobes. The glands arise as an outpocketing of the vagina and are well supplied with tracheae. The interior wall of each gland is lined with many thin, bifurcate projections around and between which many bacteria are to be found. The glands which open at the vaginal orifice are compressed during egg laying to coat the egg with secretion. Other distortions of the female abdomen also tend to squeeze the glands.

We decided to isolate and culture the bacteria; so the colleterial glands were carefully dissected from young and old female beetles which had been partially sterilized by surface washing in 70% ethanol. The whole glands were washed in sterile water and torn apart in a drop of water in a sterile Petri dish. The bacteria were streaked on to plate count agar (Difco) in Petri dishes and incubated at 21° C for 72 h. The resultant colonies were predominantly of one type, which seemed to confirm that the bacteria originated from within the glands rather than from surface contamination.

A liquid medium, containing yeast extract (2.5 g/l.), tryptose (Difco, 5.0 g/l.) and dextrose (1.0 g/l.), was inoculated with the bacterial isolate. After 5 days of incubation at 21° C the culture was bioassayed for grass grub beetle attractant activity in field conditions. Simple vane traps of the type described before⁵, each containing approximately 5 ml. of the culture medium, attracted male grass grub beetles. Traps containing medium alone or which were unbaited were unattractive. The bacteria were thus clearly capable of producing an insect sex attractant chemical. Similar levels of attractant activity have been obtained with dilute aqueous solutions of phenol.

The bacterial culture was centrifuged, extracted with chloroform and examined by thin-layer chromatography on silica gel in the systems: chloroform and hexane: ether (90:10). After spraying with diazotized sulphanilic acid prominent spots were obtained with R_f values similar to those of phenol (0.31, 0.12, respectively). A second phenolic material (R_f 0.95, 0.24) which was present in small quantity did not seem to be associated with attractant activity.

The conversion of tyrosine to phenol by bacteria is well known and it is possible that a similar pathway is followed in this case.

We thank Mr J. Boyd for technical assistance.

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Steroids and Squalene in *Methylococcus capsulatus* grown on Methane

THE ubiquitous occurrence of steroids in nature, and their fundamental importance for plant and animal life, are well known. Until 1967, steroids had been encountered only in eukaryotic organisms, and had not been detected in prokaryotic organisms, the bacteria and blue-green algae. In recent years, sterols and the sterol precursor, squalene, have been detected in several classes of such organisms (Table 1). Quantitative studies are few, but they indicate that the amounts of steroids (expressed as a percentage of dry weight of organism) found in the prokaryotes are substantially lower than those of eukaryotes. Here we report that the bacterium *Methylococcus capsulatus*, grown on methane as the sole carbon source, contains comparatively large amounts of squalene and sterols.

Methylococcus capsulatus was grown in a chemostat on a mineral salt medium with a methane-air (1:1) supply. The harvested cells were extracted with chloroform-methanol and the extract was fractionated by thin-layer chromatography (TLC) on silica gel. Subsequent gas-liquid chromatography of fraction A (hydrocarbons) revealed the presence of squalene and a series of alkanes from C₂₀-C₃₀, all in approximately equimolar amounts. An additional peak ($I_{230}^{0V-1} = 3150$) was identified by mass spectrometry as another triterpene hydrocarbon: molecular ion m/e 410, base peak m/e 69, intense ions m/e 189 and 191. No squalene 2,3-oxide could be detected, but a compound co-chromatographing with farnesol was observed.

Three sterol fractions (B, C and D respectively), comprising 4,4-dimethyl, 4-methyl and 4-desmethylsterols, were obtained. Fraction B, examined by GC-MS, had two principal components (I_{250}^{0V-17} about 3510 and 3570). The first produced a mass spectrum in which the molecular ion (m/e 414) was the base peak. An ion at m/e 301 was consistent with loss of a C₈H₁₇ side chain. The second chromatographic peak yielded a mass spectrum in which the base peak was at m/e 412. The predominance of the ion at m/e 69 suggested the presence of a Δ^{24} double bond. The gas chromatographic retention indices, the retention index difference between the two peaks, and the mass spectrometric data were all consistent with structures 4,4-dimethyl-5 α -cholest-8(9)-en-3 β -ol and 4,4-dimethyl-5 α -cholesta-8(9),24-dien-3 β -ol respectively. There was insufficient sample for further investigation.

Fraction C contained substantially more material. GC-MS indicated two main incompletely separated peaks, I_{250}^{0V-17} about 3410 and 3480. The mass spectra paralleled those recorded for fraction B: the first component had a molecular ion at m/e 400 as the base peak, and the second showed m/e 69 as the base peak, with the molecular ion (m/e 398) 77% as intense. The corresponding trimethylsilyl ethers were better separated (I_{250}^{0V-17} 3315 and 3385). The first, preponderant component gave a mass spectrum in which the molecular

Table 1 Squalene and Sterols in Prokaryotic and Eukaryotic Organisms

Bacteria	Squalene percentage of dry weight	Sterols percentage of dry weight
<i>Methylococcus capsulatus</i>	0.55	0.22
<i>Halobacterium cutirubrum</i> ¹	0.1	—
<i>Staphylococcus</i> sp. ²	0.002	none
<i>Rhodococcus vannielli</i> ³	+	—
<i>Rhodospirillum rubrum</i> ³	+	—
<i>Rhodospirillum rubrum</i>	0.0001	none
<i>Azotobacter chroococcum</i> ⁴	—	0.01
<i>Streptomyces olivaceus</i> ⁵	—	0.0035
Blue-green algae		
<i>Phormidium luridum</i> ⁶	trace	0.003
<i>Anacystis nidulans</i> ⁷	—	+
<i>Fremyella diplosiphon</i> ⁷	—	+
Eukaryotic organisms		
<i>Polystictus versicolor</i> (Basidiomycete) ⁴	+	0.1
<i>Aspergillus nidulans</i> (filamentous fungi)	0 to 0.03	0.6-0.9
<i>Debaryomyces hansenii</i> (yeast)	0.003	+

Presence is indicated by +, lack of information by —. The second set of data on *Rhodospirillum rubrum* and those on *Debaryomyces hansenii* and *Aspergillus nidulans* are the unpublished observations of J. M. Lynch and W. W. Reid.

ion (m/e 472) was the base peak, both at 70 eV and 20 eV electron energy. The second component yielded a molecular ion at m/e 470, amounting to 78% of the abundance of the base peak at m/e 69. The retention data and the full mass spectrum were in close agreement with those recorded for authentic 4 α -methylzymosterol trimethylsilyl ether.

The combined data for fraction C are therefore compatible with the assignment of structures 4 α -methyl-5 α -cholest-8(9)-en-3 β -ol and 4 α -methyl-5 α -cholesta-8(9),24-dien-3 β -ol to the two principal components. Similar mass spectra would probably be obtained from the Δ^7 and $\Delta^{8(14)}$ -isomers, but the Δ^7 -isomers would have distinctly longer retention times and are clearly not present as major components.

Fraction D has not yet been examined by GC-MS because of lack of material. TLC properties of the major component of this fraction suggest that it is 5 α -cholesta-8(9),24-dienol (zymosterol). No evidence could be obtained by TLC, GLC or GC-MS for the presence of either lanosterol or cycloartenol in fraction B.

Stanier has suggested that there may be a significant quantitative difference between the steroid contents of prokaryotes and eukaryotes⁸. At the moment, *M. capsulatus* seems to be unique in that its biosynthetic pathway from squalene is restricted to zymosterol derivatives. Another unusual feature is the large pools of squalene and 4 α -methylsteroids. Finally, the detection of the other triterpene hydrocarbon excites speculation about its biosynthetic status.

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Effect of Activated Charcoal in Agar on the Culture of Lower Plants

Proskauer and Berman¹ have described a technique for culturing green organisms such as filamentous algae and moss protonema on an agar substrate containing activated charcoal which may simulate conditions found in nature. They ascribed the resulting morphological changes primarily to a decrease in the amount of light transmitted by the blackened agar, and considered their technique a simulation of natural soil conditions. We have performed similar experiments which also show that moss development is altered on charcoal-agar, but which require another interpretation.

The moss, *Funaria hygrometrica*, was grown on an artificial substrate (modified Knop's solution supplemented with A-Z solution and EDTA complex in 2% agar²). The agar was supplemented with various concentrations of activated charcoal, 4% MnO₂ (crystalline limonite) or soil).

On charcoal-agar a series of characteristic alterations in development were observed². The most noteworthy were: a limited development of protonema, acceleration of bud formation and the absence of a spreading caulonema on the agar surface. In addition to the "fairy ring" of gametophores typical for this type of moss, the cultures frequently (with suitable illumination up to 100% of the protonema) developed a single bud in the middle of the protonema. This last phenomenon occurred only by direct culture on agar containing 0.05% or more charcoal. The lower concentrations resulted in only a slight darkening of the medium. This phenomenon

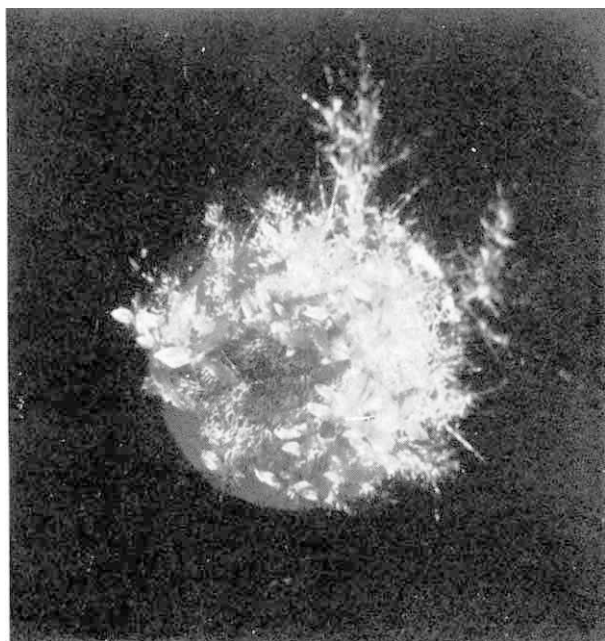


Fig. 1 Protonema from *Funaria hygrometrica* grown on colourless agar surrounded by charcoal-agar. The protonema is inhibited, while bud formation is enhanced by the adsorption through the charcoal. $\times 5$.

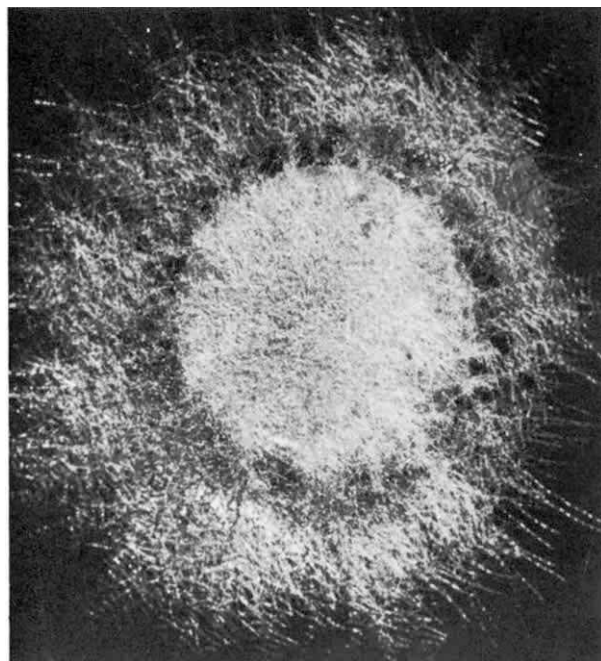


Fig. 2 Protonema of *Funaria hygrometrica* of the same age as in Fig. 1 grown on colourless agar surrounded by MnO₂-agar. Note the spreading of the protonema over the MnO₂-agar; bud formation has not yet started. $\times 5$.

was not observed on limonite or soil-agar, nor did it occur on charcoal-agar covered with colourless cellophane. All these morphogenetic changes were observed when charcoal-agar was covered with a layer of colourless agar up to 4 mm thick. All the changes except central bud formation occurred when the protonema were cultivated on a colourless agar disk as much as 28 mm in diameter which was separated by a glass wall from charcoal-agar in the rest of the Petri dish (Fig. 1).

When limonite was employed, which absorbed nearly as much light as charcoal, bud formation was barely accelerated and the protonema spread out in the same manner as on colourless substrate (Fig. 2). The alterations in development, therefore, cannot be ascribed to light absorption by the agar—which, incidentally, leads also to a change in the relative humidity of the substrate surface. Our results are best interpreted if one assumes that activated charcoal adsorbs substances which influence the development of the protonema *in vitro*. For central bud formation, these substances pass through the substrate; for the other changes, through the gas phase of the Petri dish from where they are also adsorbed. Because it is known that the development of moss protonema is regulated for the most part by substances which are liberated into the substrate by the protonema themselves³, the adsorption of such substances by activated charcoal is not surprising⁴. Our results do not disprove that in nature, adsorption of these substances onto soil particles may play a role in moss development.

Although our observations support those of Proskauer and Berman¹, who based most of their work on the rhizoid system of mosses, they further indicate that adsorption may be a critical factor, and that charcoal-agar simulates more than just natural light conditions.

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BOOK REVIEWS

Growth of an Industry

The Chemical Industry 1900-1930: International Growth and Technological Change. By L. F. Haber. Pp. x+452. (Clarendon: Oxford; Oxford University: London, February 1971.) £6.00.

MR HABER is well known for his work on the nineteenth-century chemical industry and the present volume continues the history of the industry down to 1930. It provides a valuable addition to the literature on the subject and usefully complements the recent study of ICI made by W. J. Reader. It is well written, extensively researched and largely free from complex technical jargon. But one gets the impression that the author has perhaps been a little too ambitious in trying to write what is virtually a history of the world chemical industry. As a result the book does not really fulfil its purpose and consequently it suffers from certain defects.

In the introduction, Haber sets for two chief objectives: these are to explain the more rapid and sustained growth of chemicals compared with that of other industries, and to apply economic concepts to that development. But in neither case does he really succeed. The second aim is not very explicitly formulated but, in any case, apart from a rather belated reference to Rostow's "Stages Theory" in chapter 11 (which is not a very apt choice anyway), he scarcely acknowledges the existence of theoretical or economic concepts, let alone links these to the growth and transformation of the chemical industry. Nor do the chief determinants of growth come across very clearly, for no formal model of the growth and structural transformation of industry is ever formulated. Thus he places considerable emphasis on government stimulation of chemicals, especially dyestuffs, during the First World War, and on the role of research in developing new products. There are in fact two whole chapters on research in industry and the universities, and though in each case there is an impressive amount of detail, not a great deal is done to illustrate just how this fostered the growth of the industry. When we come to the

question of technology, it is even less apparent how this influenced the industry's performance, for we are told that for much of the period supply consisted predominantly of traditional lines, notably alkalis, acids, dyestuffs and fertilizers. Demand factors are very inadequately treated. But the real problem is not the absence of growth determinants but the failure to analyse them carefully enough in relation to each other.

It might have been more fruitful if Haber had confined his investigations to the chief chemical producers, namely the US, Germany, Britain and France. After all, these countries accounted for some 75 per cent of world chemical output both before and after the war, and most of the other countries he covers only accounted for quite minute amounts. By including a wide range of small producers in his survey he merely adds to his task without giving more than a potted summary of the developments in these countries. Had the latter been excluded it might have been possible to devote more time to analysing in greater depth the main issues faced in the chief producing nations.

The result, I feel, of this attempt at wide geographical coverage is that some of the important issues are inadequately explored. For example, the important field of explosives is almost wholly ignored. To be fair, the author does acknowledge this omission at the start though he never defines very precisely just what he intends to cover in the volume. Second, though considerable information is given about cartels, agreements and mergers, very little attempt is made to estimate their impact. Third, the statistical basis of the book is rather weak. On numerous occasions Haber stresses the fact that good statistical data are hard to come by and this we can accept, though one cannot help thinking that greater effort in this direction, for example by using company records, might have proved to be more rewarding. Moreover, confidence in his handling of statistics is not enhanced when we glance at pages 174 and 320 and find that he uses two different definitions of the US chemical industry and thereby confuses the issue regarding the weight-

ing of the latter in total world output. This inconsistency arises partly through not defining clearly the coverage of the industry at the start.

It is also surprising to find 1930 selected as the terminal date. The author justifies this on the grounds that the proliferation of products after the world depression would complicate the story too much. This hardly seems a very adequate explanation. That notwithstanding, it is certainly very irritating to be cut short at 1930 when so many exciting things were happening, not only on the technological front, but also in the economic field. Thus we hear little about how chemicals fared in the slump, how international agreements stood the test of excess capacity and falling prices, or what effects transpired (in terms of efficiency and so on) from the great mergers of the 1920s, notably those of ICI and IG Farbenindustrie. No doubt Haber intends to continue the story in a later volume, but I cannot help feeling that 1939 would have been a much more suitable year on which to end the present volume.

Writing about the chemical industry is not the easiest of tasks and the author has clearly sifted an enormous wealth of detail and arranged it in coherent order. But whether he makes the best use of it is another matter. Certainly there are still many unknown quantities on which we require further information. The chief impression that emerged from the book was the way in which Germany dominated the field both before and after the war (taking a narrow definition of the industry), and the way in which other countries struggled to catch up. Certainly by the post-war period Germany had lost some of her lead, but quite what happened earlier is difficult to say because of the absence of aggregate data. Nevertheless, Germany still retained the edge on other countries especially in the matter of research. It would seem, however, that we were already conscious of this situation before the appearance of Mr Haber's book so that in effect what he has done has been to put some flesh on our skeletal framework of knowledge of the industry.

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Threat or Blessing?

Nuclear Power and the Public. Edited by Harry Foreman. Pp. xviii + 273. (University of Minnesota: Minneapolis; Oxford University: London, March 1971.) £4.25.

THIS book consists of a series of papers presented at a conference held at the University of Minnesota in 1969. The motivation for this conference lay in the public concern felt about the possible effects on the environment of a high megawattage nuclear power plant being built 40 miles up the Mississippi from the twin cities of Minneapolis and St Paul. Prior to this, the issues at stake in allowing the Northern Power Company to proceed with building the nuclear plant had aroused widespread concern within the United States and the aims were to present the facts about environmental pollution arising from nuclear power plants for the benefit of all. The symposium brought together scientists working in the field of radiation effects together with experts and recognized authorities in all fields relating to the problem of generating power from nuclear reactors.

It is a fact that the consumption of electric power in the United States has doubled every decade since 1940. The long term predictions are for this rate of power consumption to continue at an ever increasing rate. It had become apparent that some alternative to fossil fuels as a source of power would be needed, and the answer seemed to lie in nuclear power. The ever increasing concern about maintaining the state of the environment, however, has led people to question whether this form of power generation is creating a larger problem than it is solving. The conference proceedings present the facts but by and large the reader has to make up his own mind whether or not the advantages of nuclear power outweigh the disadvantages.

One of the most striking aspects of the proceedings is the lack of agreement between the two factions that are seen to emerge. The authorities concerned with the design, building and planning of nuclear power plants continuously make the point that all nuclear power plants in the United States more than conform to Government regulations concerning the pollution of the environment with radioactive and other waste. The conservationists argue, convincingly, it must be said at times, that some of the regulations and maximum permissible levels set down are far from adequate to protect the public.

The final conference papers deal with the benefits and risks of nuclear power. They are delivered by proponents of both sides of the argument, Dr Barry Commoner, professor of plant physio-

logy at Washington University, St Louis, and James T. Ramsey, a commissioner of the US Atomic Energy Commission. Commoner, who introduces the concept of a benefit/risk ratio, points out that the standards of safety set by the Federal Radiation Council (FRC) are based on the conclusion that the risks of radiation exposure are proportional to the dose of radiation received. Therefore as there is no intrinsically safe level, all standards set by the FRC require judgment of how large a risk should be taken by the population in return for the value gained from the activity that caused the exposure. Ramsey makes the point that the benefits of nuclear power are great in terms of low cost and conservation of resources and also, compared with fossil fuel plants, beneficial in conservation of the environment. On what common grounds do the proponents meet? There is indeed some measure of agreement; both feel that it is up to the public to study the facts and make judgments based on them. Ramsey also feels that the scientific community should play a more important role in assisting the public in this regard.

All the papers presented in the book contain some facts and figures that are directly applicable to the problem of environmental pollution. The conservationists put forward their case with conviction but unfortunately seem to be operating in a vacuum with no relation to the power needs of the United States. The representatives of the governmental agencies seem to shield behind nationally and internationally recognized radiation standards. There is obviously need for cooperation on inquiries into the viability of radiation standards.

For anyone with even a passing interest in the subject the book is well worth reading. On the basis of the facts found within the thirteen papers, a reasoned judgment can and should be made on whether the present standards of safety set when building nuclear power stations are adequate, or whether a new and more stringent set of standards are required that would in all probability increase the cost of power generation. ALUN JONES

Migraine Described

Migraine: The Evolution of a Common Disorder. By Oliver W. Sacks. Pp. 298. (Faber and Faber: London, January 1971.) £2.50.

THE mechanism and underlying causes of migraine remain elusive, despite a growing volume of research which has already produced some tantalizing clues. Indeed, a comprehensive theory of causation is still lacking; one which

would account for all the clinical manifestations of this widespread and disagreeable disorder, as well as for the precipitation of attacks by dietary, hormonal, emotional, visual and other stimuli—or apparently by none at all.

Dr Sacks aims to fill this gap, and in his book gives his personal construction of the psycho-biological substructure of migraine. The early chapters describe the various clinical forms and presentations of migraine (with many illustrative case histories) and discuss these in the light of the author's particular interests. The core of the argument, however, lies in the section on "The Basis of Migraine". Sacks interprets the observed and experienced phenomena of the migraine attack as being essentially manifestations of a slow excitation-inhibition-excitation cycle of electrical activity arising within the brain; "a form of centrencephalic seizure, the activity of which is projected rostrally on the cerebral hemispheres and peripherally via the ramifications of the autonomic nervous system" (p. 212), thus accounting for the variety of symptomatology. The circumstances which provoke the attack, together with certain of the neurological prodromata, are seen as the initial excitation. The inhibitory phase occupies the principal part of the attack, manifested by withdrawal of the patient into his suffering and eventually into sleep; an accompanying excess of parasympathetic activity being responsible for vomiting and for the vasodilatation which is the immediate cause of the headache and which may be localized. Finally, the sense of well-being accompanying recovery is seen as the next phase of excitation.

The author points out that a biological basis for such a reaction does exist in the possibility of response to threat by withdrawal and conservation, rather than by fight and flight. These two responses seem to be innate in man as well as in animals. Migraine attacks are classified as paroxysmal—due primarily to neuronal instability with little external provocation, circumstantial—resulting from excess of external or internal physical stimulation—and habitual—in which the stress is some emotion, experienced as dangerous, and in which the migraine attack may also have a symbolic content.

This makes an agreeably comprehensive theory, but is Sacks right? It is difficult to know, for the evidence which he presents in support of his views is scanty. This evidence rests entirely on his interpretation of the migraine attack as the result of waxing and waning of excitation of brain structures, and on the importance which he places on the significance and nature of those rare clinical states, involving particularly the aura, which lie on the borders of both

migraine and epilepsy. "Deep in the brainstem . . . is the origin of the migrainous process, slow tonic changes of excitation and inhibition; but the detection of these changes and the demonstration of their nature and cause, have completely eluded us . . ." (p. 207). As he freely acknowledges, the author has reevaluated older theories of the origin of migraine, as expounded particularly by Liveing and by Hughlings Jackson; but in the light of his own extensive clinical experience, and interpretation of the literature. The style is characterized by many quotations, from Aretaeus to Wittgenstein.

At times he is unjustifiably dogmatic and he tends to do less than justice to viewpoints with which he doesn't agree—for example, he underrates the importance of current work on the biochemical mechanisms, in which the most practical prospects for rational therapy probably lie. His discussion of psychoanalytic aspects is unnecessarily complex and indeed is out of date.

This is not a book with which to begin a study of migraine and it is not a satisfactory source book of scientific information on the subject.

RITA HENRYK-GUTT

Hit and Miss

Molecular Radiation Biology: The Action of Ionizing Radiation on Elementary Biological Objects. By Hermann Dertinger and Horst Jung. Translated by R. P. O. Hüber and P. A. Gresham. Pp. x+237. (Longman: London; Springer-Verlag: New York and Berlin, March 1971.) £3.00.

THERE are at any given time a number of fashionable words and phrases, the judicious use of which may attract attention to a piece of scientific writing. A number of authors have, for example, learned that the inclusion of "DNA" in the title of a paper guarantees an extra two hundred reprint requests from perusers of *Current Contents*. Another example of a fashionable phrase, I suspect, is "molecular biology", which may explain its inclusion in the title of this book. Molecular biology, if it means anything, means the explanation of biological processes and organization in terms of molecular function and structure. If one expected *Molecular Radiation Biology* to deal very largely with the effect on biological processes and organization of molecules whose structure and function had been altered by radiation, one would be disappointed. The book deals rather with the hypotheses and facts about the absorption of energy and the production of damaged molecules; chemistry and physics rather than biology.

A more honest title would have been

"Radiation Biophysics" or "Physico-chemical Aspects of Radiation Biology". Judged in that context, the book has very much to commend it. There are excellent chapters on the primary processes of energy absorption, hit theory and target theory, including most of the important recent developments. They provide good evidence that target theory is alive and well and can still provide a unifying basis for radiation biology. Much of the progress in this field has come from the study of treatments which modify the sensitivity of biological molecules, such as oxygen, temperature, water and chemical protective agents. Although at first sight the existence of sensitivity modifiers might seem to be inconsistent with target theory, this is not so and the authors have done well to include them convincingly within the general framework of target theory. It is surprising, however, that no mention is made of the interesting developments made in the field of chemical sensitizing agents during the last decade.

There have been several attempts in recent years to characterize hypothetical radiation lesions in DNA on the basis of, for example, their reparability, susceptibility to oxygen, and differential induction with radiations of different linear energy transfer. Dertinger and Jung have made a useful synthesis of these various attempts, but it is unfortunate that they have used the terminology of one group of workers but with new meanings including those applied by other workers to a different terminology. Although self-consistent within the book, confusion could arise if readers were to go to the original literature, although failure to make proper attribution makes this somewhat difficult.

There is a substantial chapter on the effect of irradiating ribonuclease, a topic on which one of the authors has worked, and four chapters on nucleic acids and viruses. Only one of the fourteen chapters attempts to deal with the response of living things (in this case bacteria) to altered molecules of DNA, and it is one best ignored. The authors may be excused for much of it being now out of date, but not for the oversimplifications and errors it contains, revealing a superficial knowledge of the biological aspects of their subject.

It is stated that the book originated from teaching material, and as a biophysical introduction to radiation biology it is to be recommended for students rather more strongly than my reservations above might imply. The book's limitations could always be made good in other ways. As an introduction to the subject for postgraduates it is also good, but access to the more biological literature would have to be sought elsewhere. B. A. BRIDGES

Chain Reactions

Introduction to the Kinetics of Chemical Chain Reactions. By F. G. R. Gimblett. (European Chemistry Series.) Pp. viii+199. (McGraw-Hill: Maidenhead and New York, 1970.) £2.10.

It is difficult to see the justification for a book on this subject intended solely for the undergraduate. It will be the exceptional student who reads the whole book, and he will not find it particularly well balanced. For the majority, who would probably only read some sections, this is not a serious criticism.

After an introductory chapter, the author deals with the hydrogen halogen reactions and the treatment is more detailed and up to date than in other elementary texts. This chapter, which could have been an excellent one, is spoiled by some statements that are either wrong or misleading for the undergraduate reader. It is also repetitive in places (this fault recurs throughout the book). The hydrogen fluoride reaction could, with advantage, have been left out.

Gimblett then continues with a discussion of linear chain reactions involving free radicals. It seems to me misguided to introduce the Rice-Herzfeld mechanism by a discussion of the poorly understood pyrolysis of *n*-hexane. The treatment of the ethane decomposition is quite good, though here and elsewhere there is the usual overemphasis on reaction order and the apparent lack of understanding that many of the unimolecular steps which occur in chain reactions do not have integral orders. Some of the suggested chain ending steps cannot be the major ones, and to quote a chain length to four significant figures would be considered a crime even for a second year undergraduate. A short venture into photochemistry suggests the author is not at home in this field.

Branched chain reactions are dealt with in a reasonably satisfactory way, and this is followed by a treatment of liquid phase reactions. This latter chapter lacks unity. Inhibition of chain reactions is an important subject and the author deals with this aspect in some detail. While it has been important historically, the inhibition of hydrocarbon pyrolyses by nitric oxide should not be treated in an elementary text.

A final chapter on experimental techniques for identification of chain carriers and the study of their behaviour is an excellent idea. It is, however, a pity that the balance is not good and once again there are misleading statements. Half the number of techniques dealt with at twice the length would have been far more helpful. SI units are not used, and in many tables the units of the "A" factors are not quoted at all.

H. M. FREY

Surveying Writ Large

Geodesy. By G. Bomford. Third edition. Pp. x+731. (Clarendon: Oxford; Oxford University: London, January 1971.) £10.00.

How does one review the third revised edition of the standard reference tome on geodesy? It is quite impossible, of course, if only because this book "aims at covering the whole field of geodesy". In any case this is not the sort of book one takes to bed for a light read; and specialists familiar with the first and second editions will already know what they want to know about the quality of this work. I shall, therefore, concentrate on what I presume readers of this review will want to know most—how this new edition of Bomford's tome differs from the previous ones.

In fact, the textural changes are quite extensive. What Bomford would have thought of at the time of the second edition (1962) as "novelties"—electromagnetic distance measurement, electronic computers, artificial satellites and the like—have revolutionized methods of geodetic observation during the past decade. In other words, new technological developments have altered the whole basis of geodesy as much as pushing back the frontiers of knowledge. Thus the traverse is now taken as having more or less superseded triangulation for new geodetic frameworks; and because electromagnetic methods are now important so are such side issues as the effects of meteorology on the velocity of electromagnetic waves. The advantages of the computer for a subject which deals with vast masses of repetitive data are, of course, obvious. And perhaps the main reason for the increased quantity of data available is the satellite. Bomford notes, however, that satellite geodesy has become such a large and expanding subject that it is not possible to give a comprehensive treatment even in a tome.

Geodesy is, of course, two things which overlap not a little—the basis of surveying and the source of important geophysical data. There is a large area of blurred definitions here; but in so far as the two aspects of geodesy are distinguishable, Bomford tends to fight shy of the geophysical. For this reason his book will be of less interest to geophysicists than it might have been, though it is not quite as black and white as that. For example, absent from the first edition but present in the third is a brief treatment of Earth tides which forms part of a much longer chapter on physical geodesy—a chapter which incidentally tends to linger over the mathematical aspects of the subject. The geophysicist interested in the physical principles involved will, however, find

more satisfactory treatments elsewhere. For geodesy as super-surveying, though, Bomford's tome is essential.

PETER J. SMITH

Survey of Sediments

Ancient Sedimentary Environments: a Brief Survey. By Richard C. Selley. Pp. xiv+237. (Chapman and Hall: London, January 1971.) £2.25.

THERE is much to be said for a book which explains ideas within a particular field in terms of "case histories" of research work and in a manner which students can readily assimilate. On the basis of the uniformitarianism principle, the author has taken ten discrete sedimentary environments and, by analogy with present-day conditions, has attempted to show how an analysis of environment may be carried out from the sediments. Following a brief introduction to environments and facies, in which the parameters and indicators of environment are touched on, each of the environments is studied in turn—river, windblown and lake deposits, deltas and shoreline deposits, reefs, flysch, and pelagic deposits.

First, characteristics of the environment existing at the present day are set down, and this account is followed by a description of an example from the stratigraphic column deposited under similar conditions. The procedures for interpretation and analysis of the "ancient" environment are then discussed, and finally a short economic assessment of the environment is given. In so doing, the book demonstrates methods by example and hence much is to be learned from a study of it. Also, because the author has used the accounts of other workers for his "case histories", a wider outlook on the subject is achieved, for there is a pool of experience from which to draw. A short chapter is included on the mathematical approaches to analysis, and the summary of environmental characteristics at the end of the book is particularly useful.

Because the organization and content of the book are so suited to beginners in the subject, it is all the more a pity that the style is disjointed. There are too many unlinked statements and successions of short staccato sentences which, while in no way detracting from the scholarship of the book, do tend to mar its clarity. Diagrams are neatly executed and all stratigraphic sections follow a standard key. Inconsistencies do occur, however, and the construction of the standard key might have been better explained. The diagrams attempting to show three dimensional representations of facies at the end of the book fail lamentably; not only are the axes shown unconventionally but there is no way of discerning into which

plane, or octant, the data indicated fall.

The fact that "the oil industry refuses to go metric" is no excuse to be unscientific, and the indiscriminate use of metres and feet further suggests a lack of attention to detail. JOHN BROOKS

Elasticity for Engineers

Large Elastic Deformations. By A. E. Green and J. E. Adkins. Second edition, revised by A. E. Green. Pp. xiv+324. (Clarendon: Oxford; Oxford University: London, December 1970.) £4.75.

IN the intervening years between the appearance of the first edition of the book and this revised text, engineers and other applied scientists have been faced with an increasing number of problems which involve large elastic displacement behaviour. This type of behaviour can arise either from the nature of the component material, for example plastics, or from the geometrical configuration of the system as encountered in shell structure design. It is also possible to have both material and geometrical configuration effects as in the case of large inflatable membrane structures. The complete analysis of large elastic deformation systems is complex and is not adequately catered for by the usual engineering theories of component and system behaviour. The more mathematically sophisticated analytical techniques required for the complete analysis of such problems are better understood by the professional mathematician. The present text, which discusses these problems, is most suited to such a person.

In more detail, this book, which for a clear understanding is dependent on the early chapters of *Theoretical Elasticity* by A. E. Green and W. Zerna, is very much the same as the first edition, with the deletion of the chapters on stability and the rheological equations of state. These subjects are omitted because of the rapid advances made during the last decade, which demand a fuller exposition in another text. Some corrections have been made to chapter II and there is an additional section on the initially curved cuboid. Also chapter VIII on thermo-elasticity has been rewritten in a way more compatible with the present state of the subject. A few additional references have been included throughout the book, keeping the reader up to date with developments in those aspects of the subject which have been covered.

The book again has a high standard of accuracy and clarity of presentation and will remain an excellent standard text on finite elastic deformation.

M. M. BLACK
H. M. SEMPLE

CORRESPONDENCE

Organochlorines

SIR,—The recent letter by Oestretcher, Shuman and Wurster entitled "DDE Reduces Medullary Bone Formation in Birds" (*Nature*, **229**, 571; 1971) prompts me to suggest that the effects of organochlorines on calcium metabolism in mammals may prove to be a fruitful line of research. During the early 1950s I was a member of a team studying the popula-

tion dynamics of a tropical pest and we were handling gamma-BHC dusts at very high concentrations in order to obtain complete kills. Several of us found that periods of field work were accompanied by a distressing de-calcification of the teeth and hastened tooth decay that we were quite unable to explain. During the past year I have wondered if the condition was due to the high doses of organochlorines that we were absorbing during

our work. If there should prove to be a relation between organochlorines and tooth structure, then the implications for all of us, especially dentists, are obvious.

Yours faithfully,

PEGGY ELLIS

64 Blenheim Road,
Caversham,
Reading

Announcements

University News

The following appointments to chairs have been made in the University of London: **Dr D. E. N. Davies**, University of Technology, Loughborough, to the chair of electrical engineering at University College; **Dr Vivian Moses**, to the chair of microbiology at Queen Mary College; **Professor D. A. Mitchison**, to the chair of bacteriology at the Royal Postgraduate Medical School.

Appointments

Mr George F. W. Adler has been appointed director of research of the **British Hydromechanics Research Association**, in succession to **Mr L. E. Prosser**.

Miscellaneous

The gold medal of the **Entomological Society of Canada** has been awarded to

Dr J. G. Rempel, former professor of biology at the University of Saskatchewan.

Ten research fellowships are offered by the **Parkinson's Disease Foundation** to medical students interested in basic or clinical research in Parkinsonism, to work for three months at their own institutions or at the Clinical Center for Parkinsonism at Columbia University College of Physicians and Surgeons. Applications should be submitted to **Dr Melvin D. Yahr**, Parkinson's Disease Foundation, 640 West 168th Street, New York, New York 10032, USA.

Entries are invited by the governors of the **Teyler Foundation** and the members of **Teyler's Second Society** for the Teyler medal. Competitors should submit a treatise, based on published results of experiments, on the morphogenetic processes leading to the formation of roots or buds from full grown cells of higher plants. Further information can be

obtained from the Honorary Secretary, Teyler's Second Society, Haarlem, The Netherlands.

The **Royal Geographical Society** has announced the following awards for 1971: Founder's medal, to **Sir George Deacon**, director of the National Institute of Oceanography; Patron's medal, to **Dr Charles Swithinbank**, British Antarctic survey; Victoria medal, to **Professor O. H. K. Spate**, Australian National University; Murchison award, to **Professor J. W. House**, University of Newcastle upon Tyne; Back award, to **Dr Claudio Vita-Finzi**, University College London; Cuthbert Peak award, to **Dr R. Murray Watson**, Royal Geographical Society's South Turkana expedition; Gill memorial, to **Dr D. B. Grigg**, University of Sheffield; Mrs Patrick Ness award, to **Major J. D. C. Peacock**, Joint Services expedition to Northern Peary Land; Cherry Kearton medal and award, to **Eugen Schuhmacher**.

Continued from p. 433.

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British Diary

Monday, April 19

HV Overhead Transmission Lines (6.30 p.m.) Mr G. Orawski, Institution of Electrical Engineers, London Graduate and Students Section, at Savoy Place, London WC2.

On the Relativity of Time (5.30 p.m.) Mr A. Trieder, British Society for the Philosophy of Science, in the Joint Staff Common Room, University College London, Gower Street, London WC1.

Semiconductor Circuit Design (vacation school, five days) Institution of Electrical Engineers, at the University College of Swansea, South Wales.

The Ethics of Population Control (8 p.m.) Mr Jack Parsons and Dr Shivaji Lal, British Society for Social Responsibility in Science, at the Institute of Contemporary Arts, The Mall, London SW1.

Tuesday, April 20

Aspects of Telemetry Under Conditions of High Acceleration (5.30 p.m., discussion) Institution of Electrical Engineers, jointly with the Institute of Measurement and Control, at Savoy Place, London WC2.

Automatic Camera Line-up in Colour Television (6 p.m.) Mr D. V. Ryley and Mrs G. Claydon, Institution of Electronic and Radio Engineers, at the London School of Hygiene and Tropical Medicine, Keppel Street, London WC1.

Better Information—Better Design (5.30 p.m., discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

Colour in Plastics (6.30 p.m.) Mr J. A. Kaitch and Mr J. E. Todd, Colour Group (Great Britain), at the University of Manchester Institute of Science and Technology, Manchester.

The Penalties of Non-Availability (6 p.m., discussion) Institution of Mechanical Engineers, Nuclear Energy Group, at 1 Birdcage Walk, London SW1.

Wednesday, April 21

Air, Fire and Water as Pollutants of Food, Society of Chemical Industry, Food Group, jointly with RSH, at Eastbourne.

Biological Applications of Particle Size Analysis (11 a.m.) Society for Analytical Chemistry, Particle Size Analysis Group, at the School of Pharmacy, University of London, London WC1.

Human Relations (7.30 p.m.) Mr Young, Oil and Colour Chemists' Association, at the Carlton Hotel, North Bridge, Edinburgh.

Integration of Miniature Circuit Breakers into Distribution Networks (5.30 p.m.) Mr H. W. Wolff, Institution of Electrical Engineers, at Savoy Place, London WC2.

Management of R & D (6.30 p.m.) Mr D. C. Dalton, Institution of Electronic and Radio Engineers, at the Central Library, Romford, Essex.

The Corrosion of Metals (6 p.m.) Dr P. J. Chilton, Royal Society of Arts, at John Adam Street, London WC2.

The Host/Implant Relationship in Orthopaedic Surgery (5.30 p.m.) Dr J. T. Scales, British Postgraduate Medical Federation, University of London, at the Institute of Dental Surgery, Eastman Dental Hospital, Gray's Inn Road, London WC1. (First of three lectures on "The Scientific Basis of Dentistry".)

Thursday, April 22

Chloroquine-Resistant Falciparum Malaria Among British Service Personnel in West Malaysia and Singapore (7.30 p.m.) Brigadier T. P. H. McKelvey, Royal Society of Tropical Medicine and Hygiene, at Manson House, 26 Portland Place, London W1.

From Microkelvin to Megakelvin Temperatures (5.30 p.m.) Professor N. Kurti, Institution of Electrical Engineers, at Savoy Place, London WC2. (62nd Kelvin Lecture).

Lipid Chromatography (6.30 p.m.) Mr S. G. Perry, Society for Analytical Chemistry, at Boots Pure Drug Co. Ltd, Pennyfoot Street, Nottingham.

Project Management, Pitfalls and Problems (6 p.m., discussion) Institution of Mechanical Engineers, Process Engineering Group, at 1 Birdcage Walk, London SW1.

Rapid Transit Vehicles for City Services (symposium, two days) Institution of Mechanical Engineers, Automobile Division, at 1 Birdcage Walk, London SW1.

Sampling (7 p.m., discussion meeting) Society for Analytical Chemistry, at the Royal County Hotel, Durham.

Friday, April 23

Aspects of the Chemistry of Fruit and Vegetable Preservation (7.30 p.m.) Mr J. D. Henshall, Society for Analytical Chemistry, jointly with the RIC, at Harris College, Preston.

Incomplete Vibrational Relaxation of Aromatic Molecules in the Gas Phase (1 p.m.) Dr J. Langelaar, Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

Light Emitting Diodes and their Utilisation (5.30 p.m., discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

Railway Automation (5.30 p.m., colloquium) Institution of Electrical Engineers, at Savoy Place, London WC2.

The Chemistry and Pharmacology of the Frusemide Diuretics (6.30 p.m.) Society of Chemical Industry, Fine Chemicals Group, at 14 Belgrave Square, London SW1.

Monday, April 26

Management of Transmission and Distribution Systems (four-day conference) Institution of Electrical Engineers, at Savoy Place, London WC2.

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